

**FIG. 1**

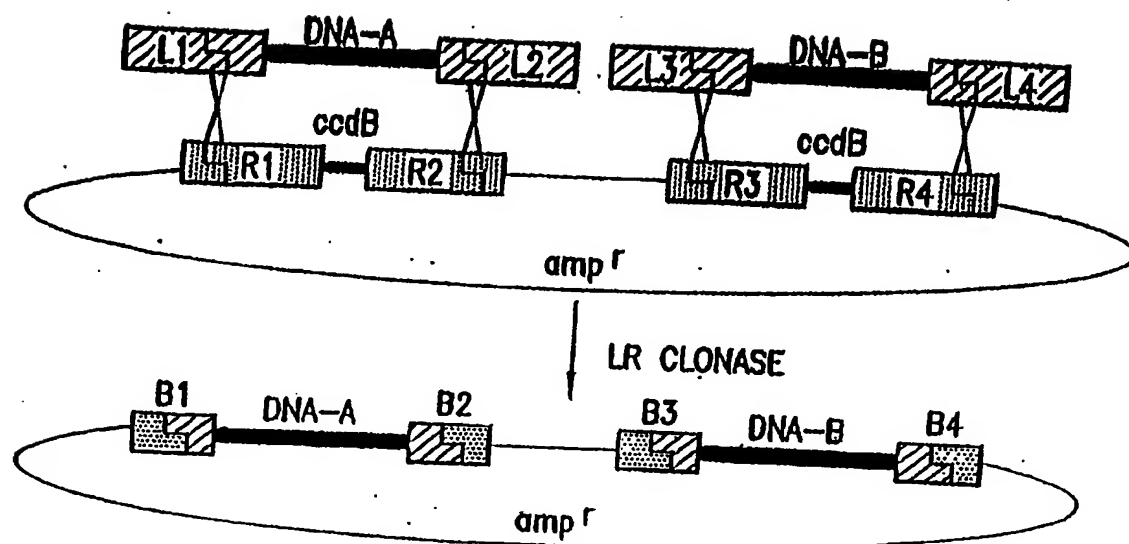


FIG. 2

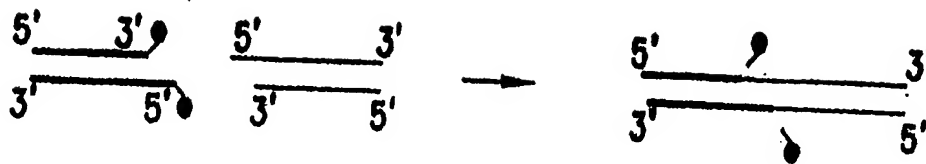


FIG. 3A

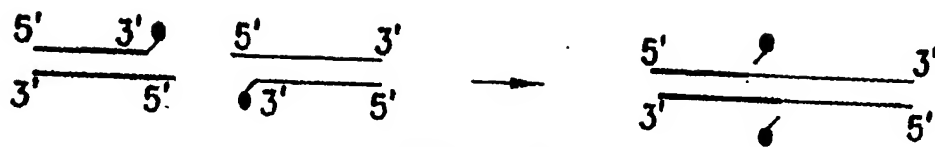


FIG. 3B

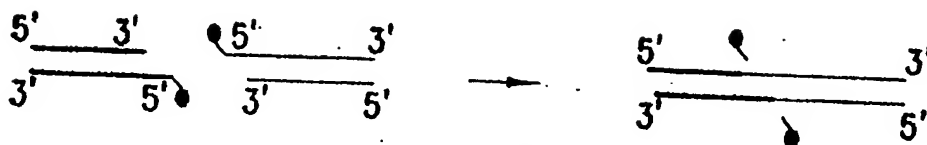


FIG. 3C

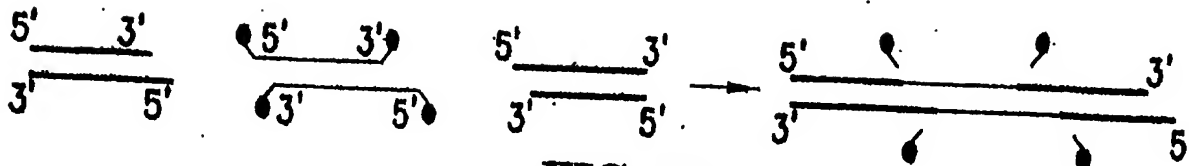
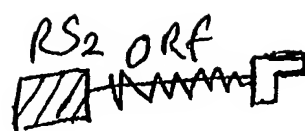
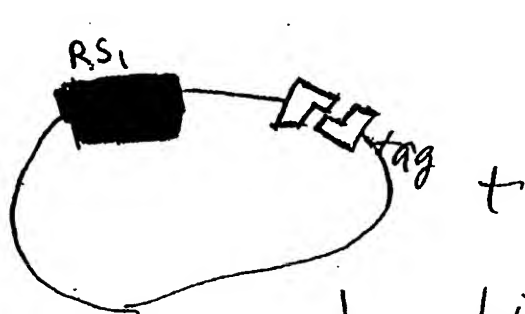


FIG. 3D



↓ RE Digest



Ligase + Recombination proteins

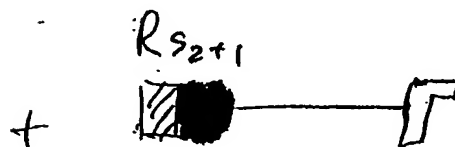
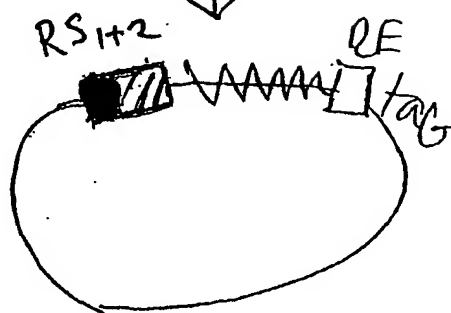


Fig. 4



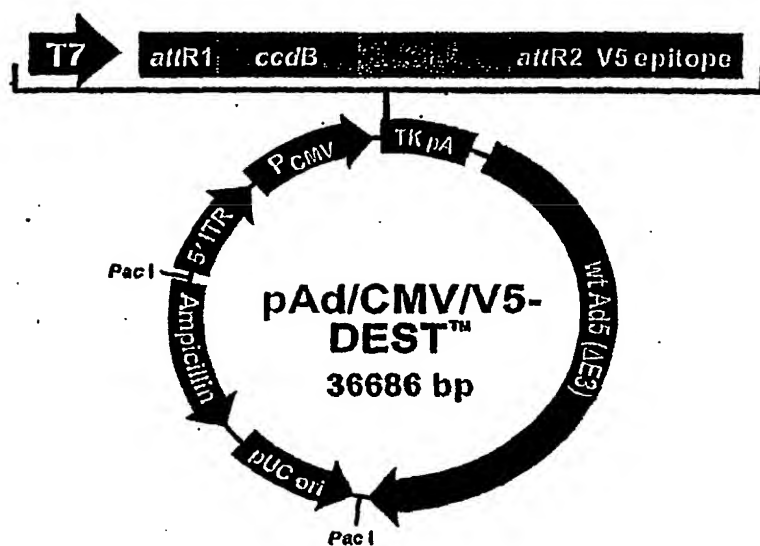
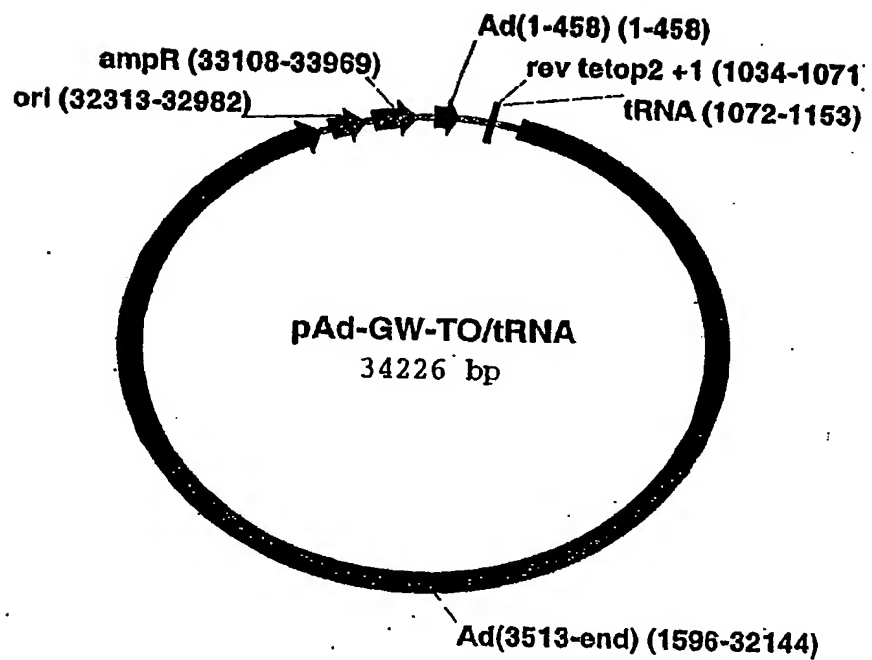


FIG. 6



**FIG. 7**

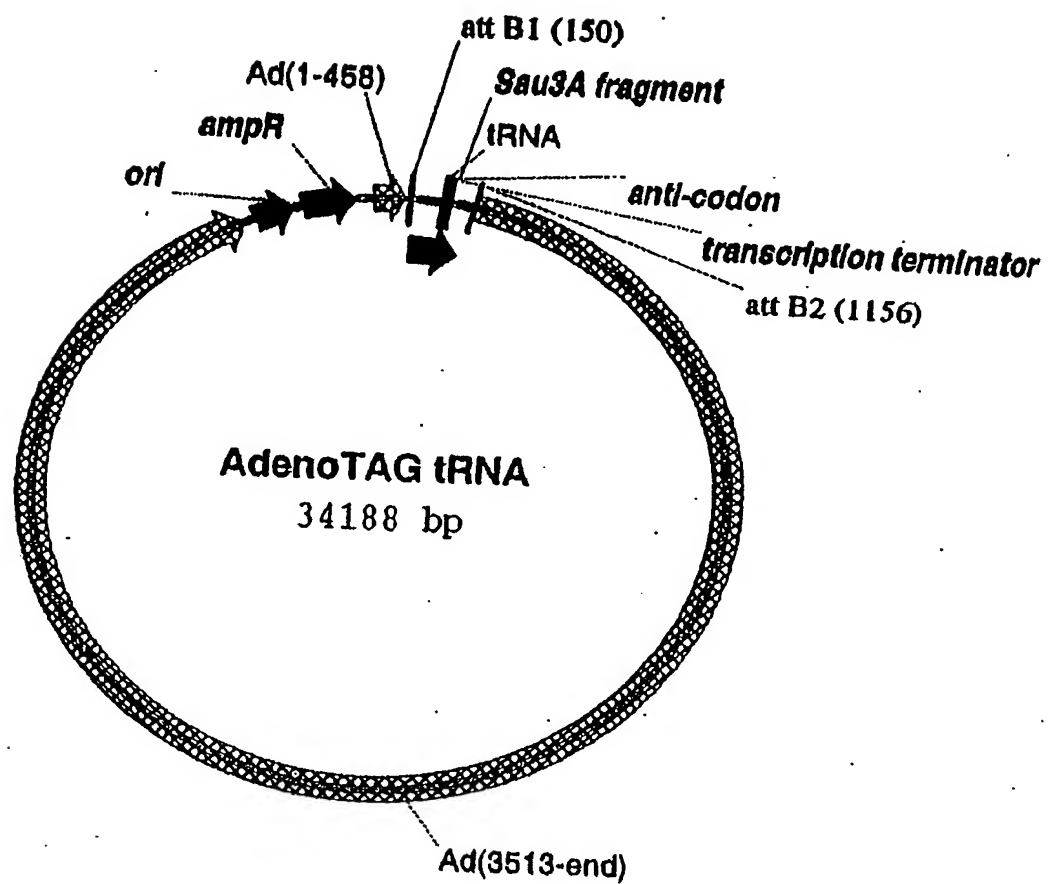


FIG. 8



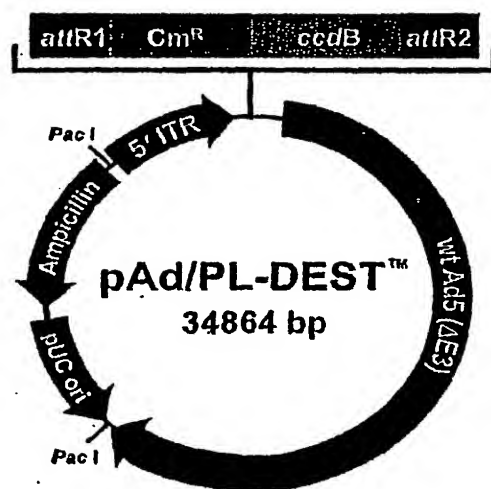


FIG. 9

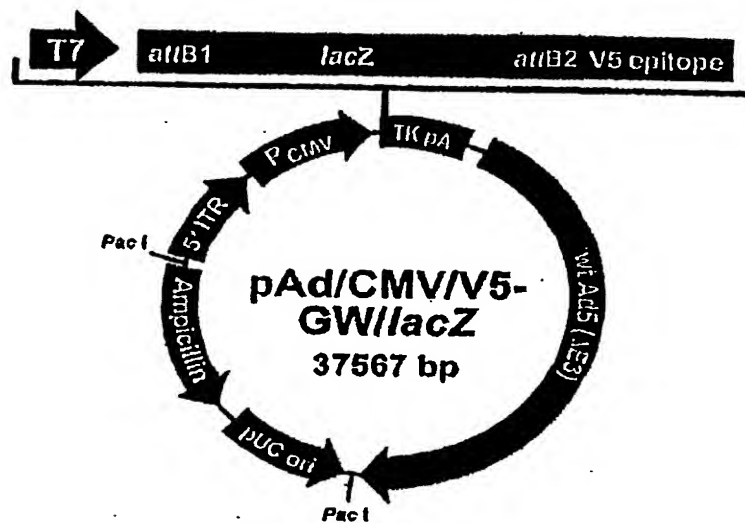


FIG. 10

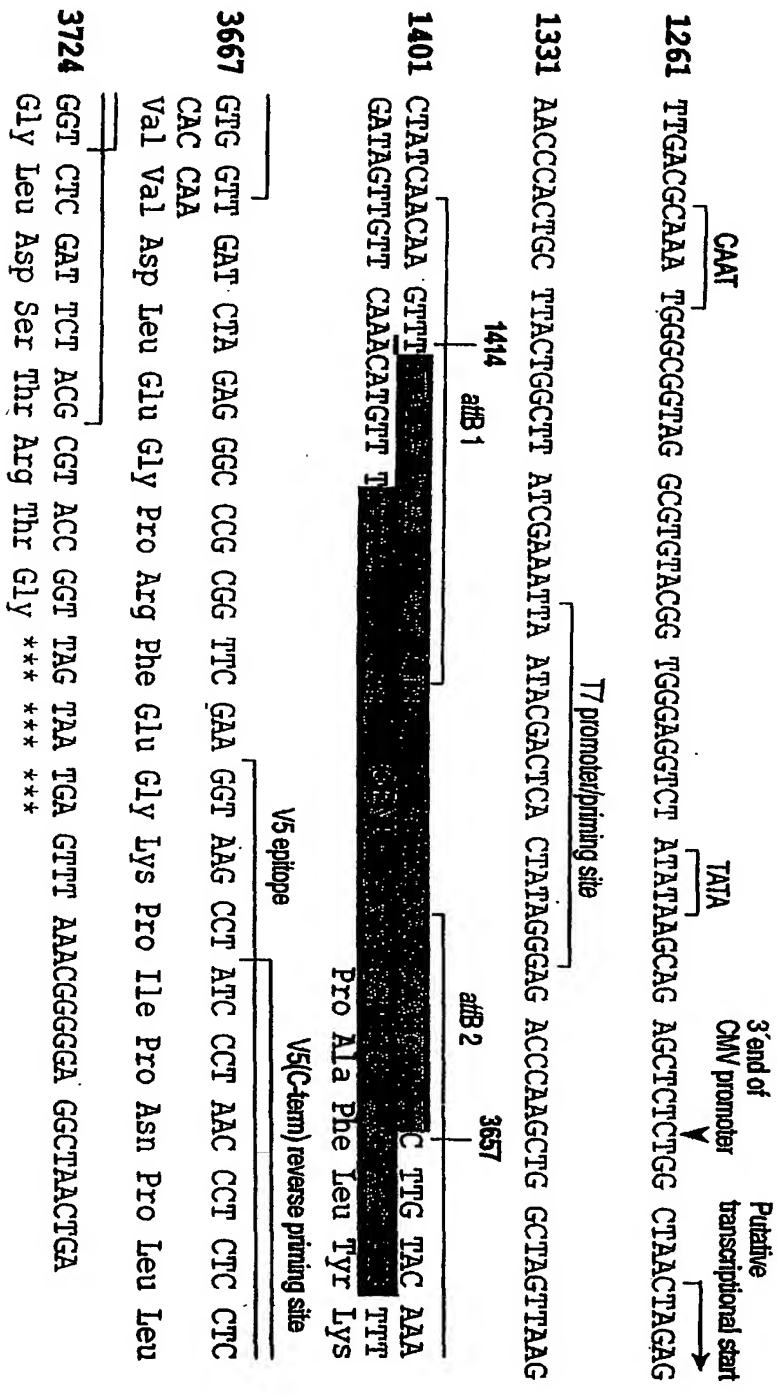


FIG. 11

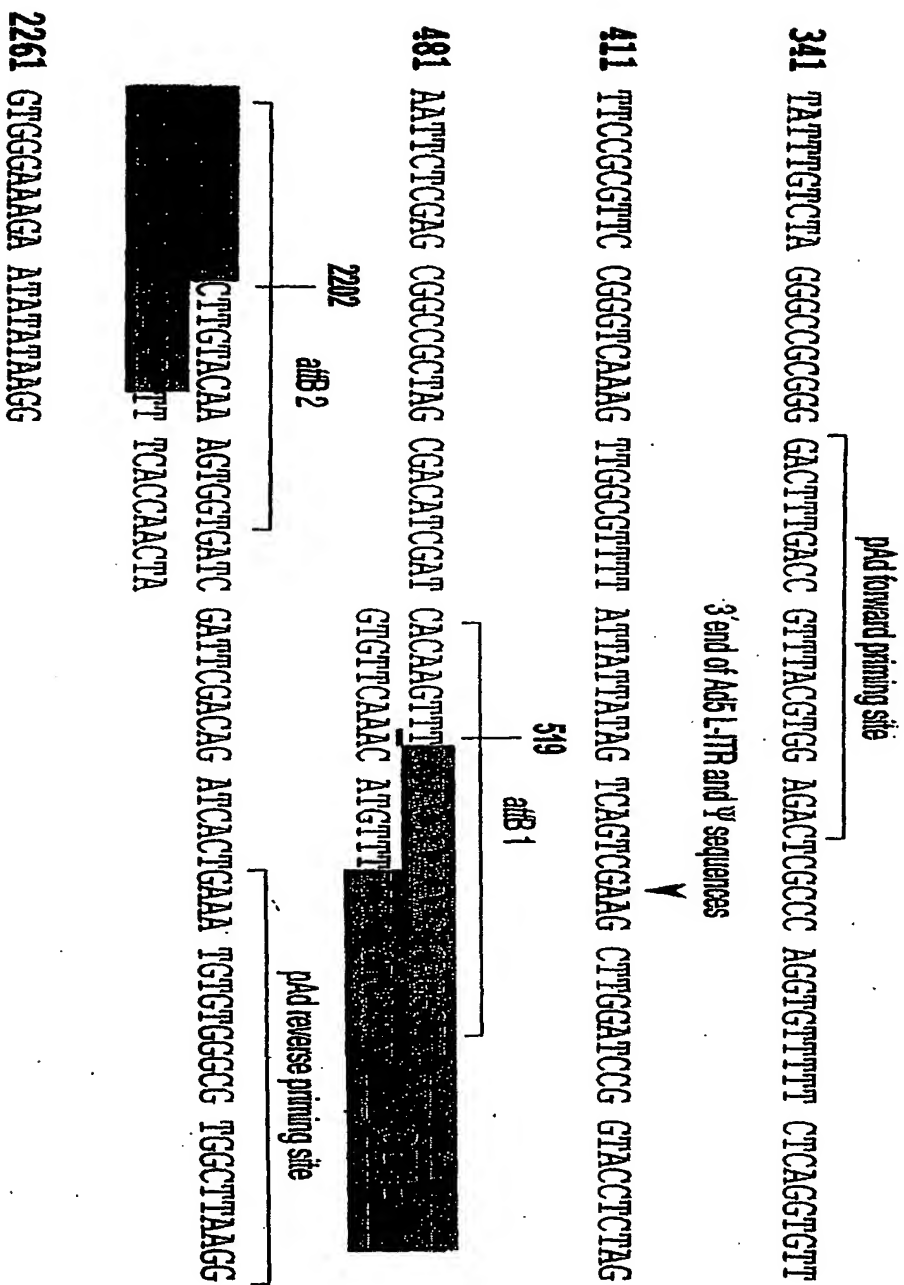
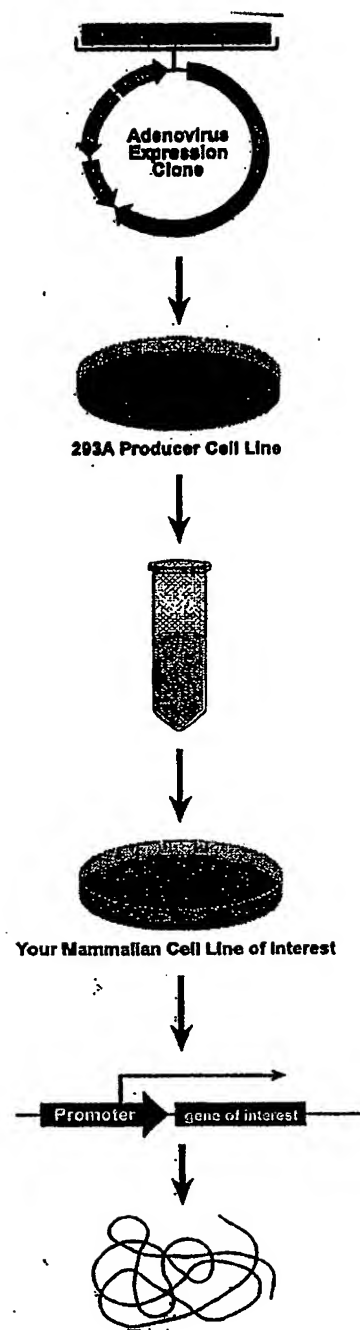


FIG. 12



**FIG. 13**



FIG. 14A



FIG. 14B

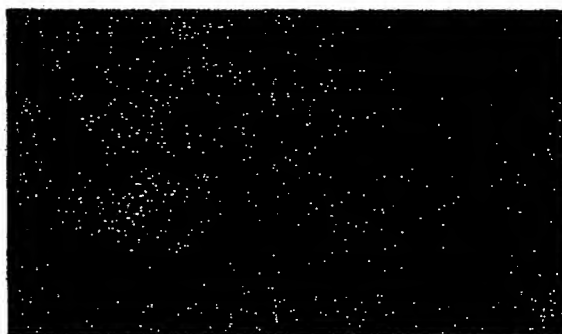
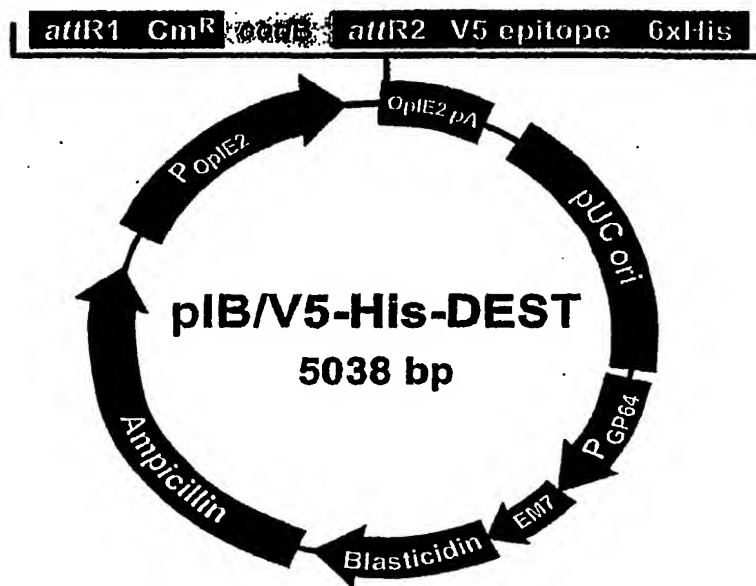


FIG. 14C



**FIG. 15**

1 GGATCATGAT GATAAACAAT GTATGGTGCT AATGTTGCTT CAACAACAAT TCTGTTGAAC  
61 TGTGTTTCA TGTGTGCCAA CAAGCACCTT TATACTCGGT GGCCTCCCCA CCACCAACTT  
121 TTTTGCACTG CAAAAAACA CGCTTTTGCA CGCGGGCCCA TACATAGTAC AAACCTCTAGC  
181 TTTCTAGAC TATTTTACAT AAATAGTCTA CACCGTTGTA TACGCTCCAA ATACACTACC  
241 ACACATTGAA CCTTTTGCA GTGCAAAAAA GTACGTGTCTG GCAGTCACGT AGGCCGGCCT  
301 TATCGGGTCTG CGTCCTGTCA CGTACGAATC ACATTATCGG ACCGGACGAG TGTGTCTTA  
361 TCGTGACAGG ACGCCAGCTT CCTGTGTTGC TAACCGCAGC CGGACGCAAC TCCTTATCGG  
421 AACAGGACGC GCCTCCATAT CAGCCGCGCG TTATCTCATG CGCGTGACCG GACACGAGGC  
481 GCCCGTCCCG CTTATCGCGC CTATAAATAC AGCCCGCAAC GATCTGGTAA ACACAGTTGA  
541 ACAGCATCTG TTCGAATTTA

TATA  
Start of Transcription

FIG. 16



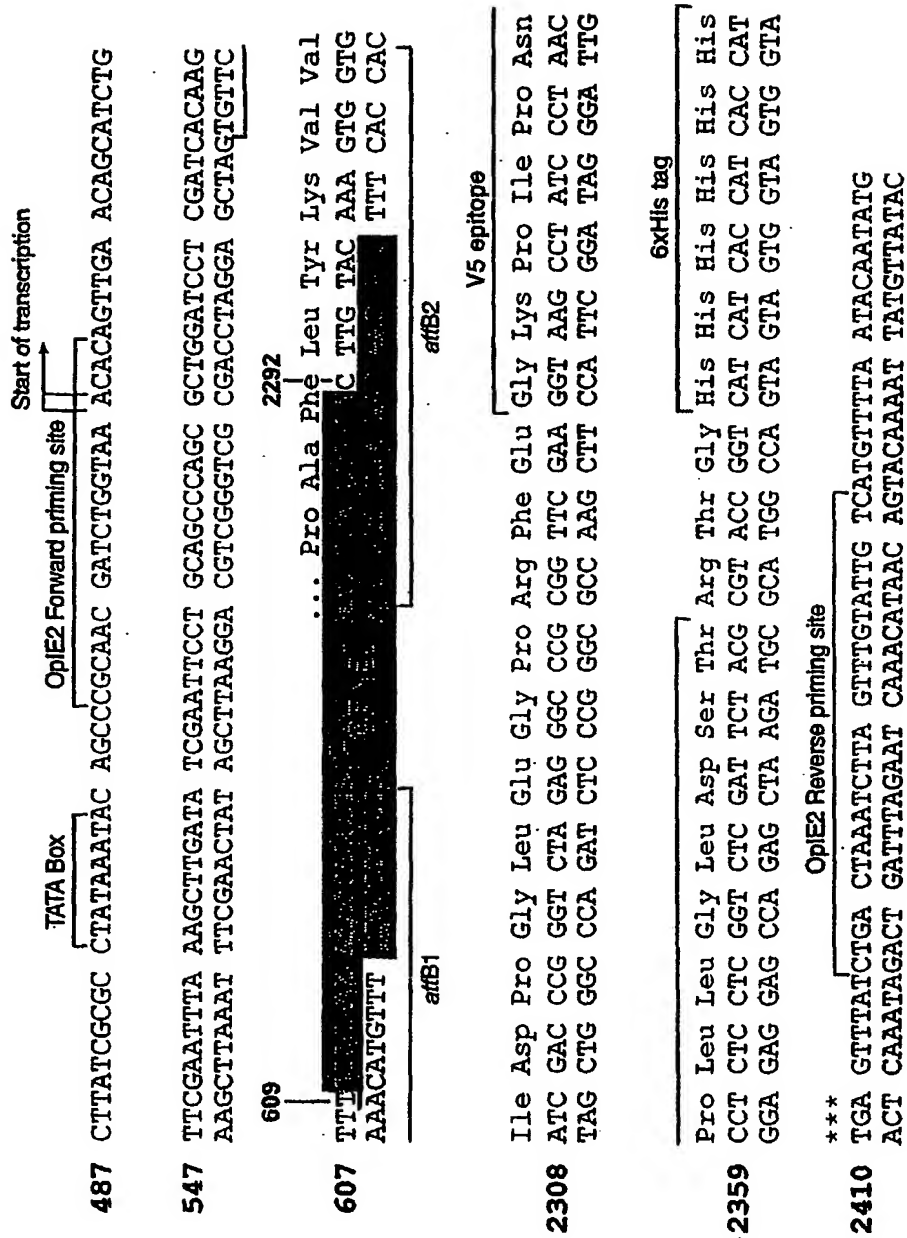
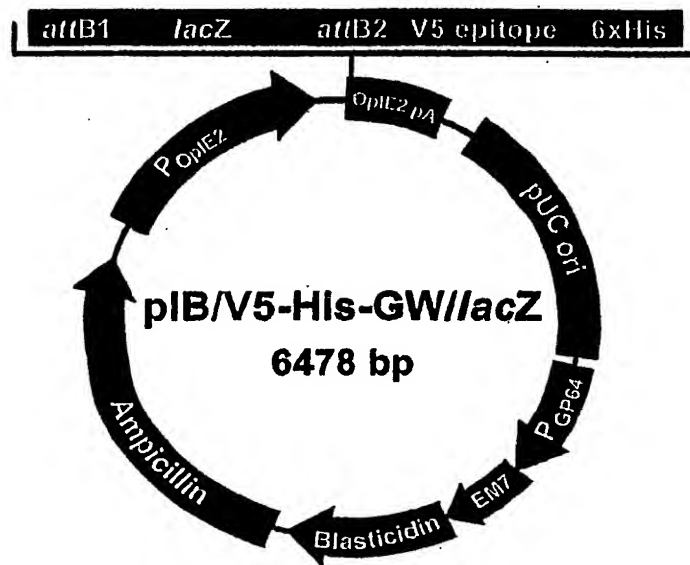
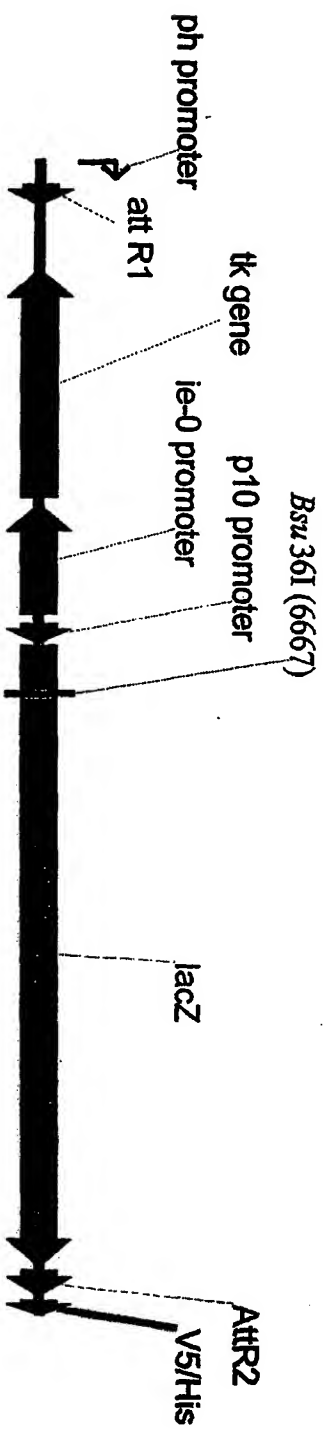


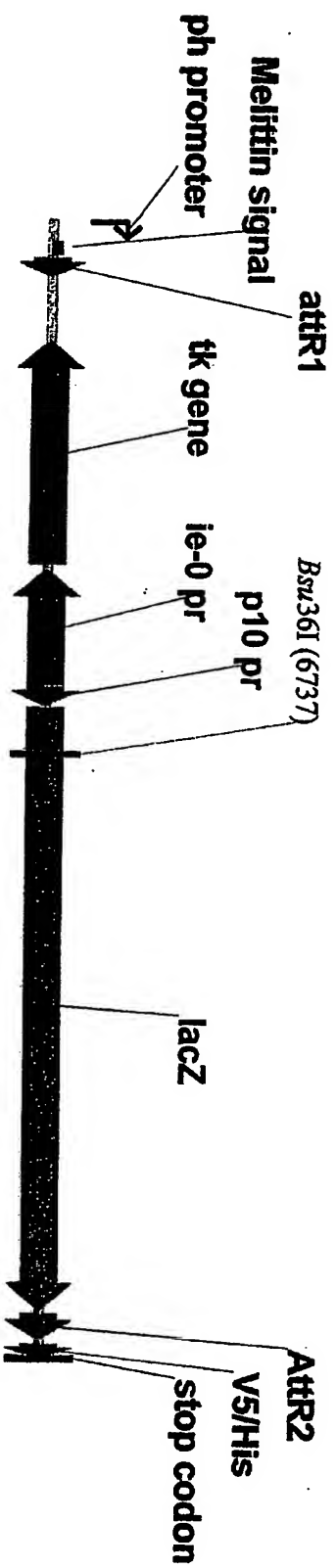
FIG. 17



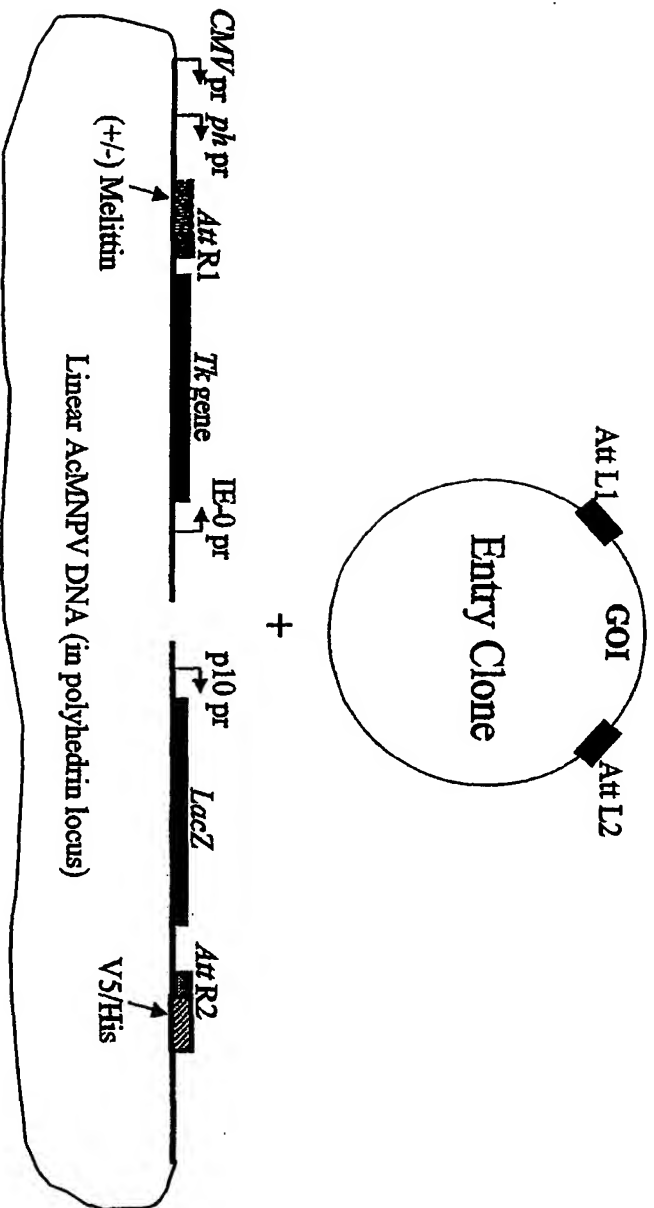
**FIG. 18**



**FIG. 19A**

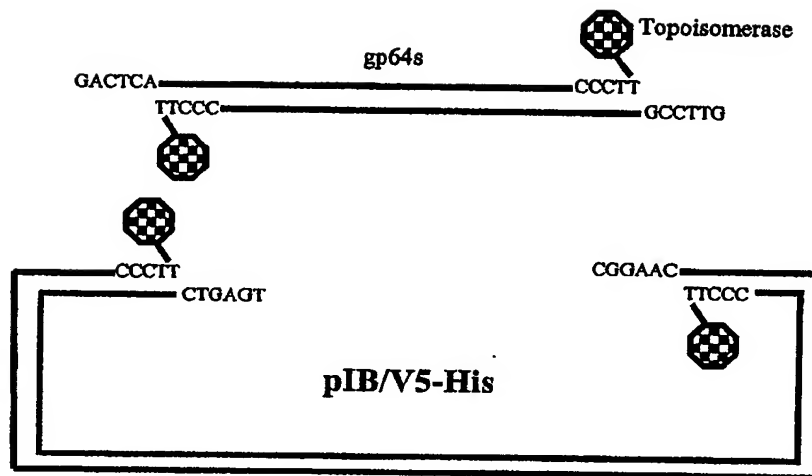


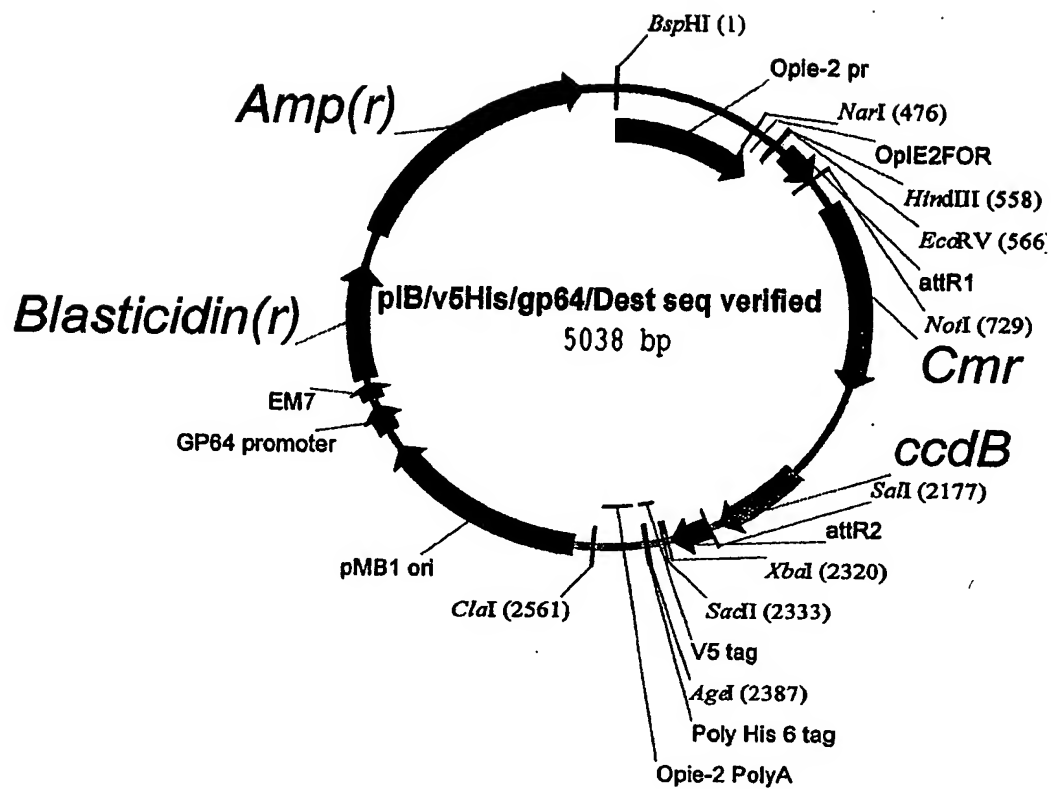
**FIG. 19B**



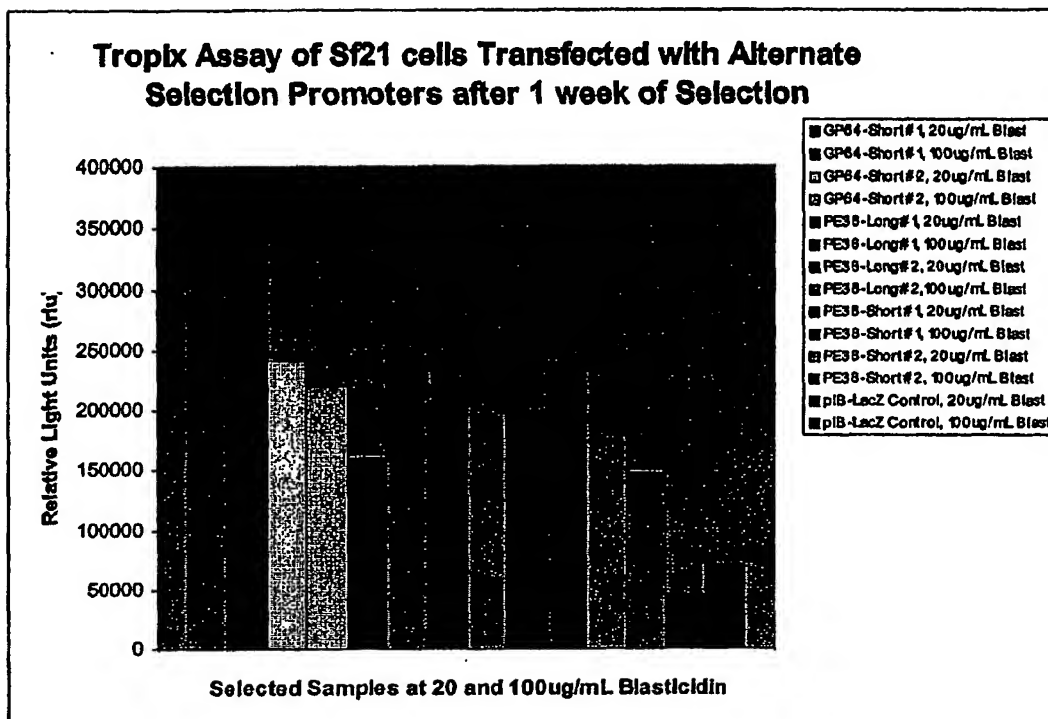
**FIG. 20**

FIG. 21





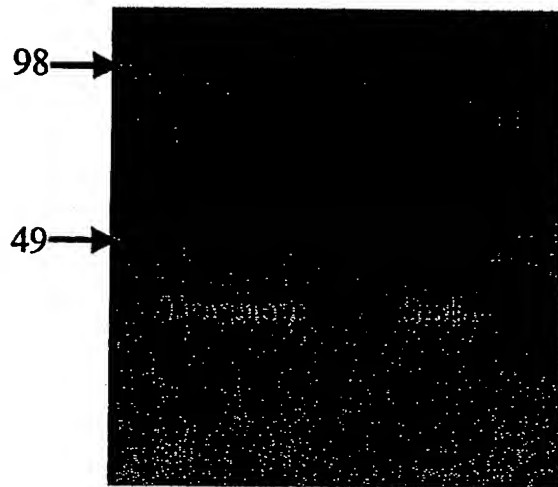
**FIG. 22**



**FIG. 23**

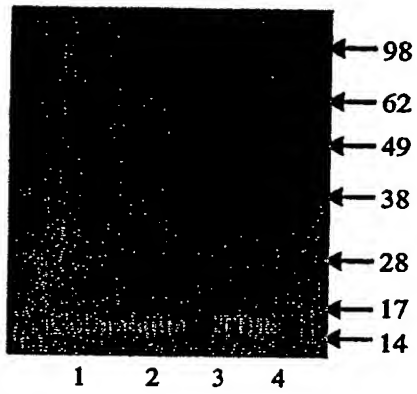


**FIG. 24**

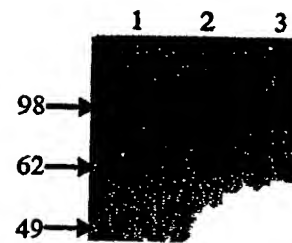


**FIG. 25**

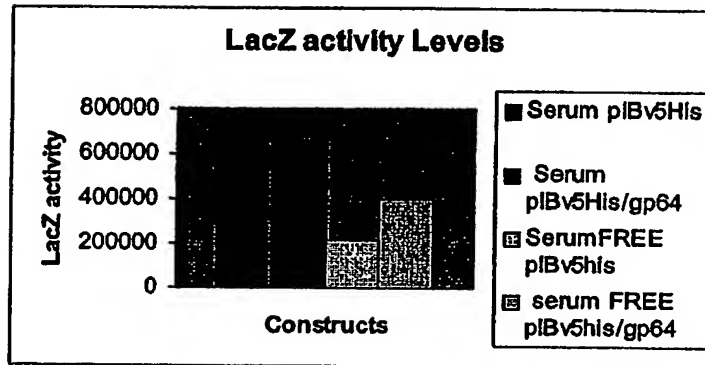
**A.**

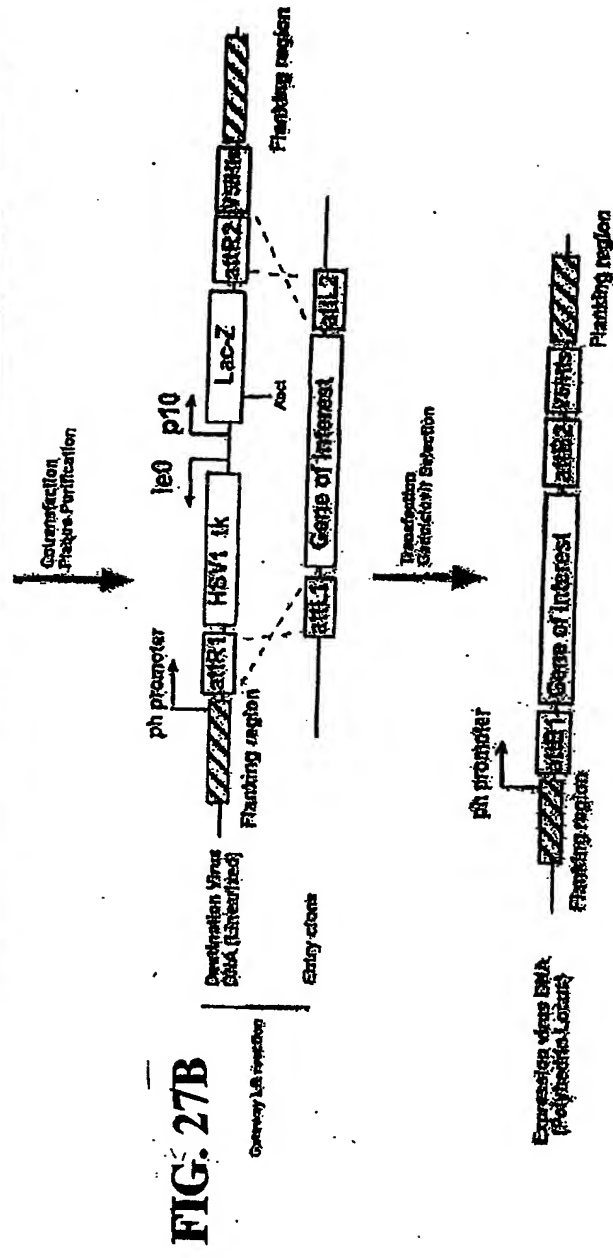
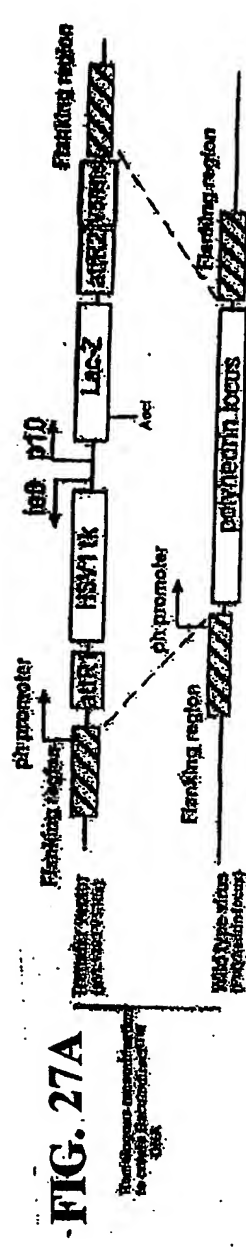


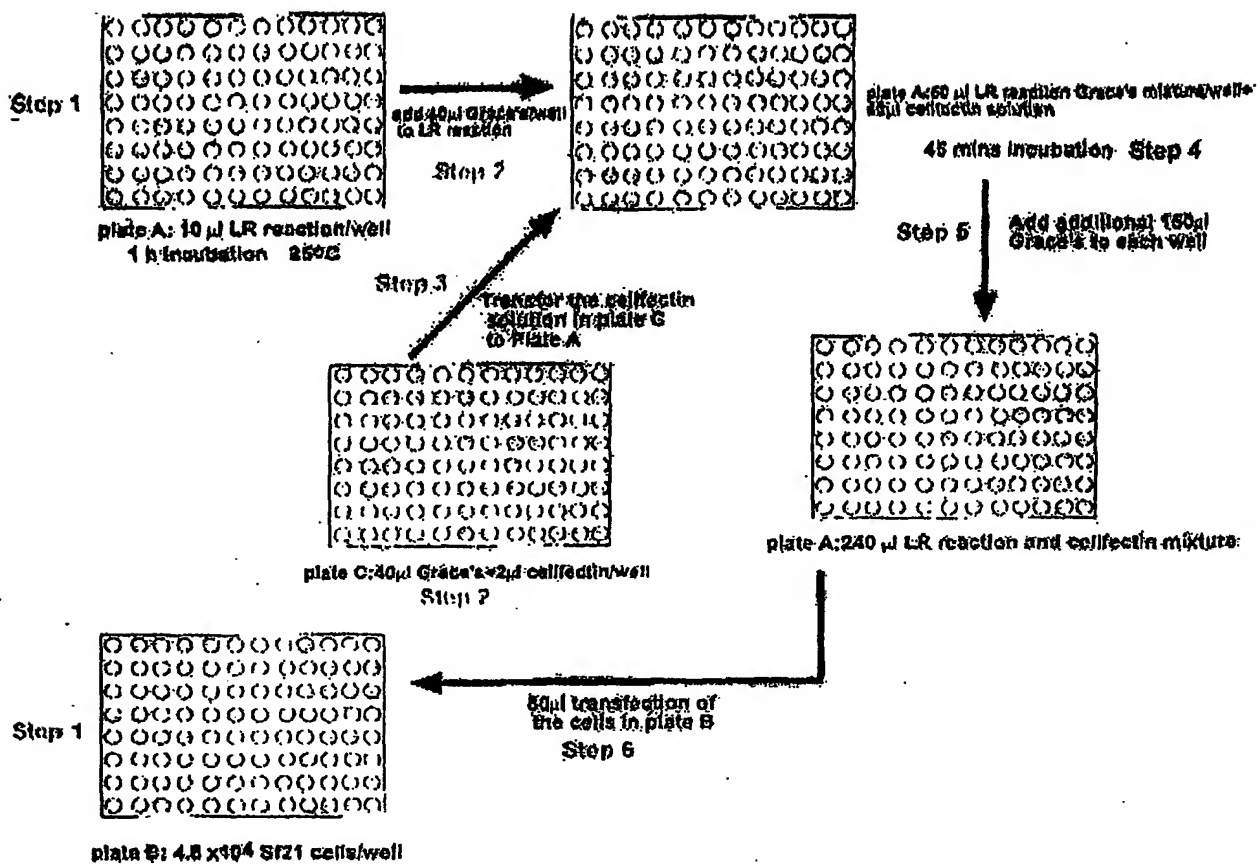
**B.**



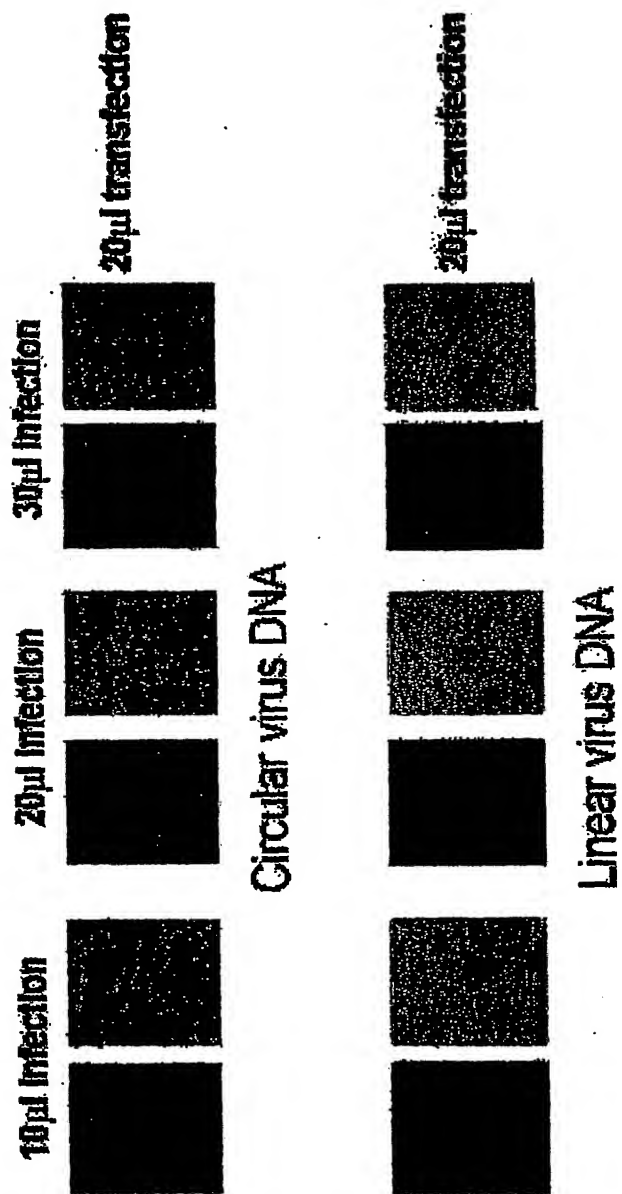
**FIG. 26**







**FIG. 28**



**FIG. 29**



FIG. 30

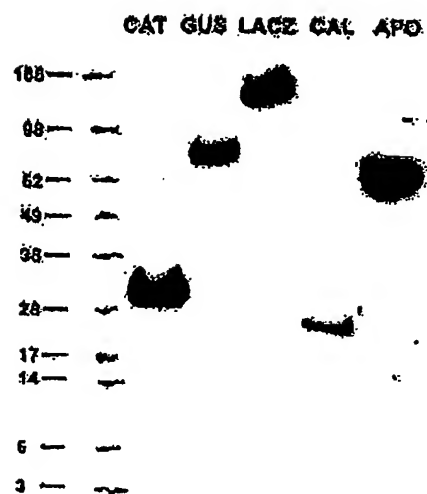


FIG. 31



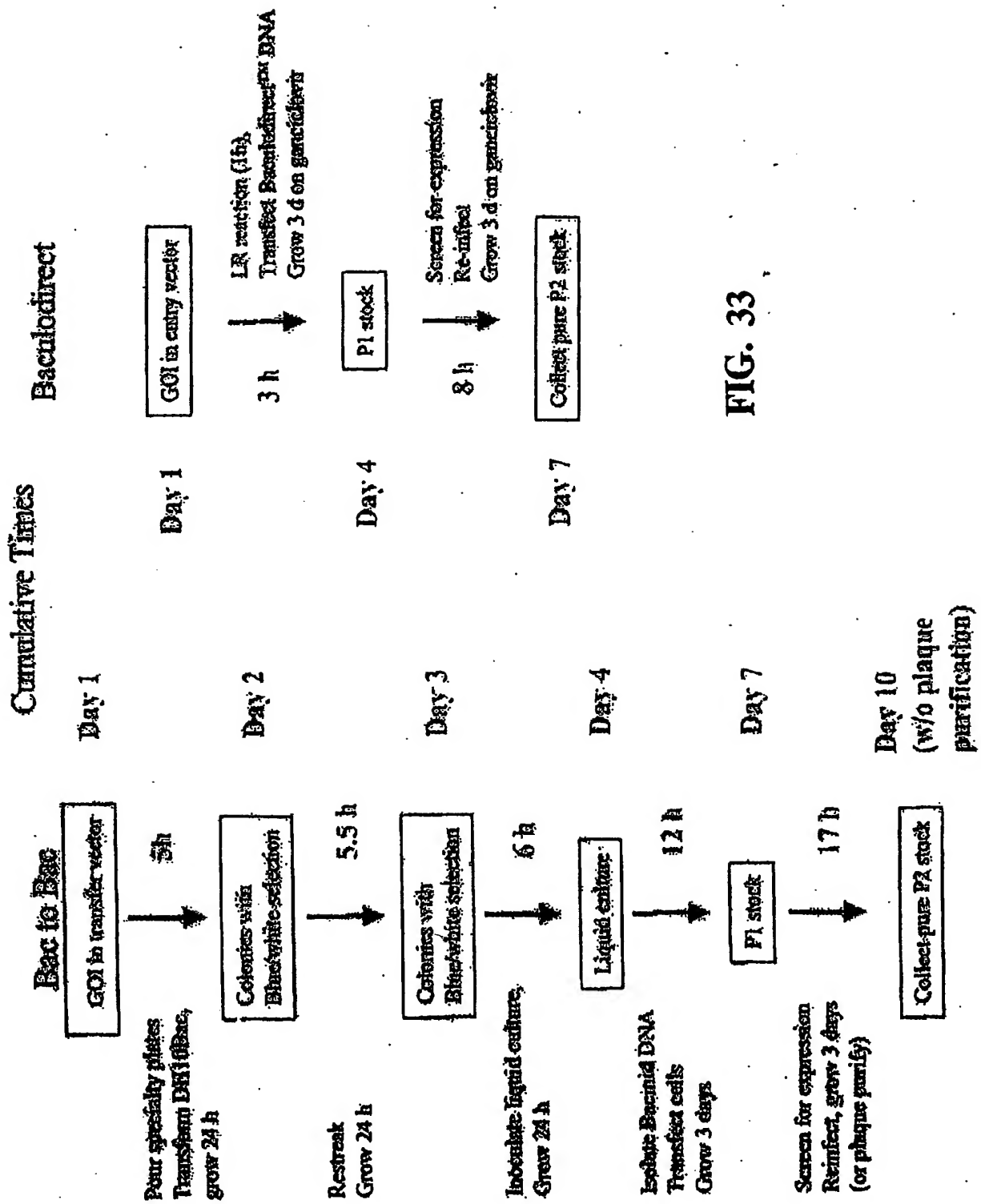
<b>Virus</b> \ <b>Titer pfu/ml</b>	<b>Transfect.</b>	<b>Infect.</b>
<b>Baculodirect™</b>	$9.8 \times 10^6$	$6.9 \times 10^8$
<b>Bac to Bac</b>	$6.9 \times 10^6$	$7.2 \times 10^8$
<b>MaxBac</b>	N/A	$3.6 \times 10^8$

Titer comparison of the three viruses by using TCID<sub>50</sub>

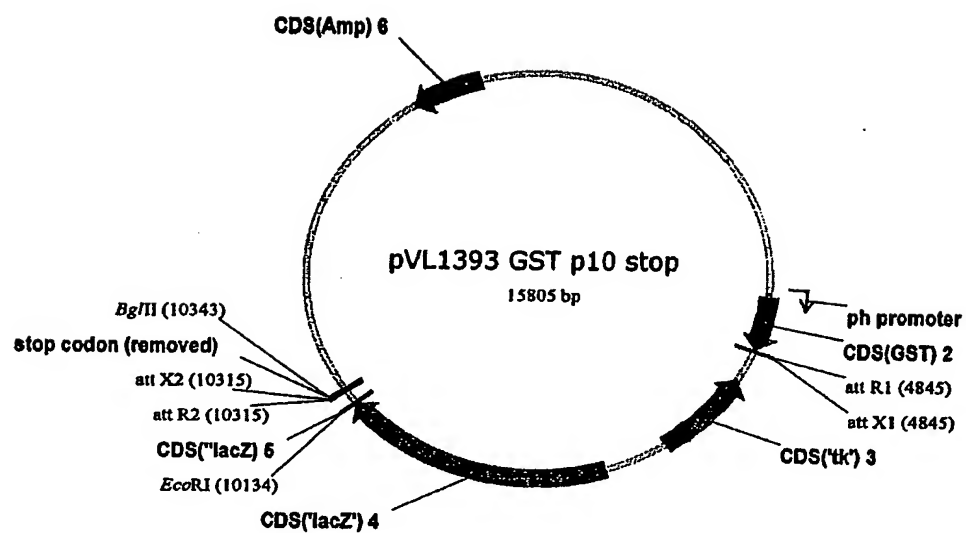
<b>Virus</b> \ <b>Titer pfu/ml</b>	<b>Transfect.</b>	<b>Infect.</b>
<b>Baculodirect™</b>	$6 \times 10^6$	$3 \times 10^8$
<b>Bac to Bac</b>	$8 \times 10^6$	$5 \times 10^8$
<b>MaxBac</b>	N/A	$3 \times 10^8$

Titer comparison of the three viruses by using plaque assay

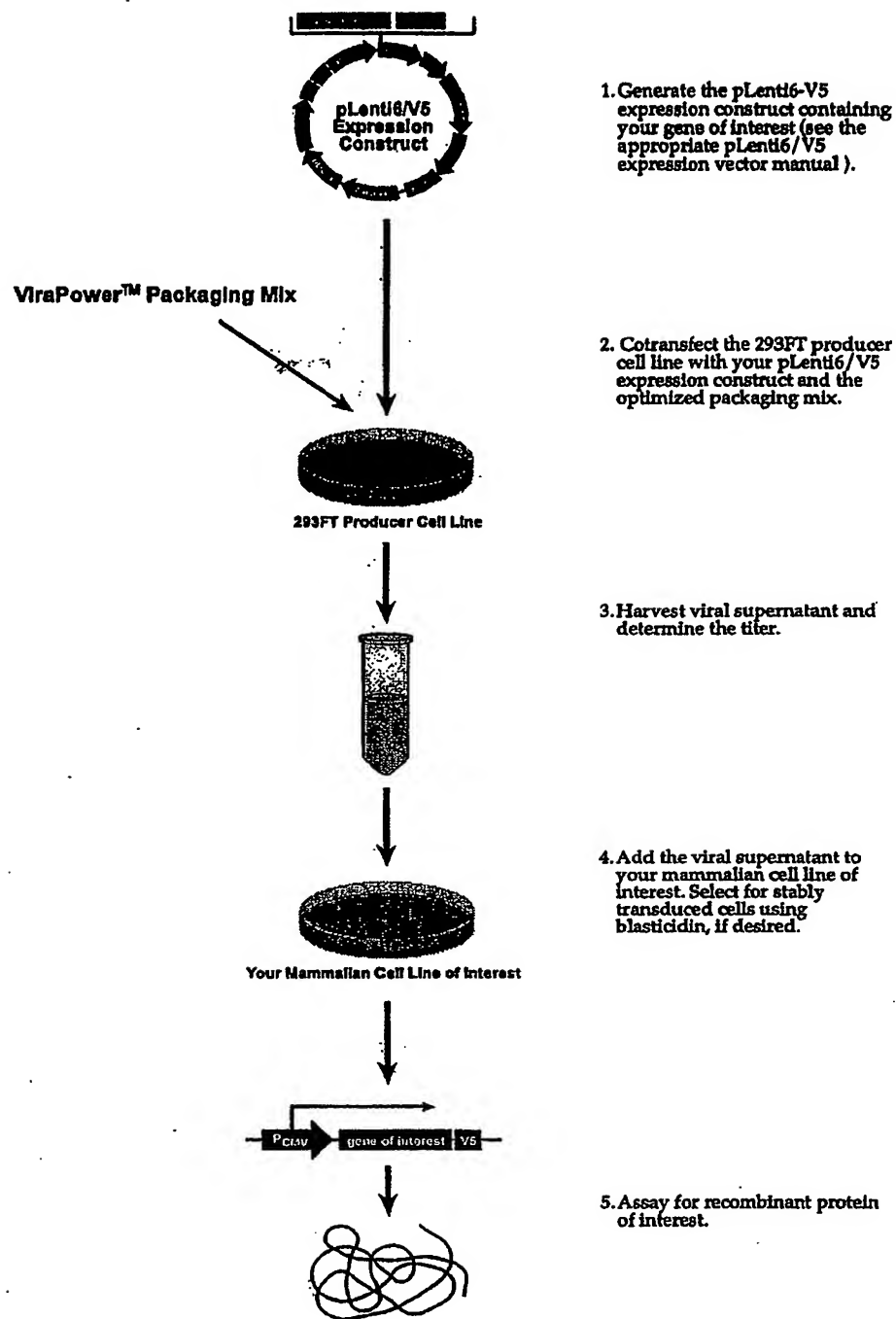
**FIG. 32**



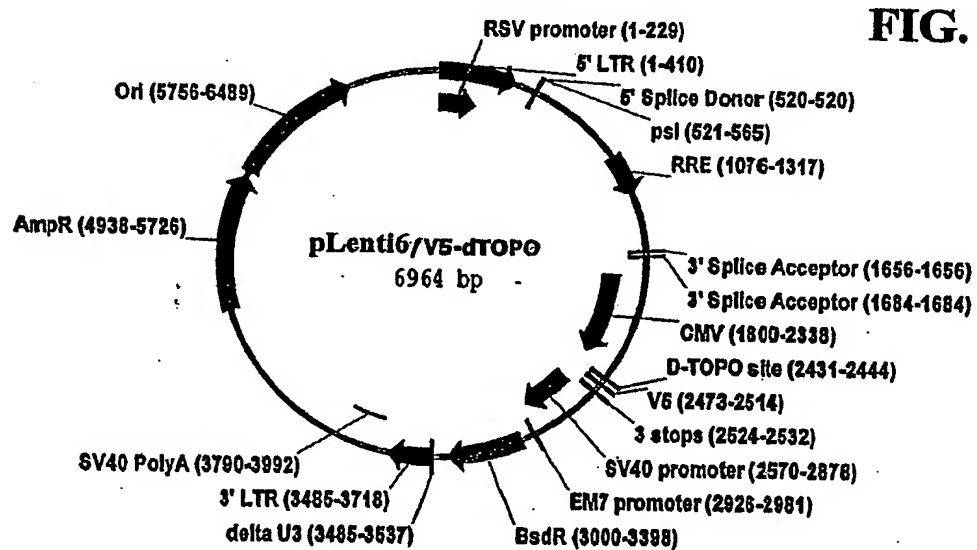
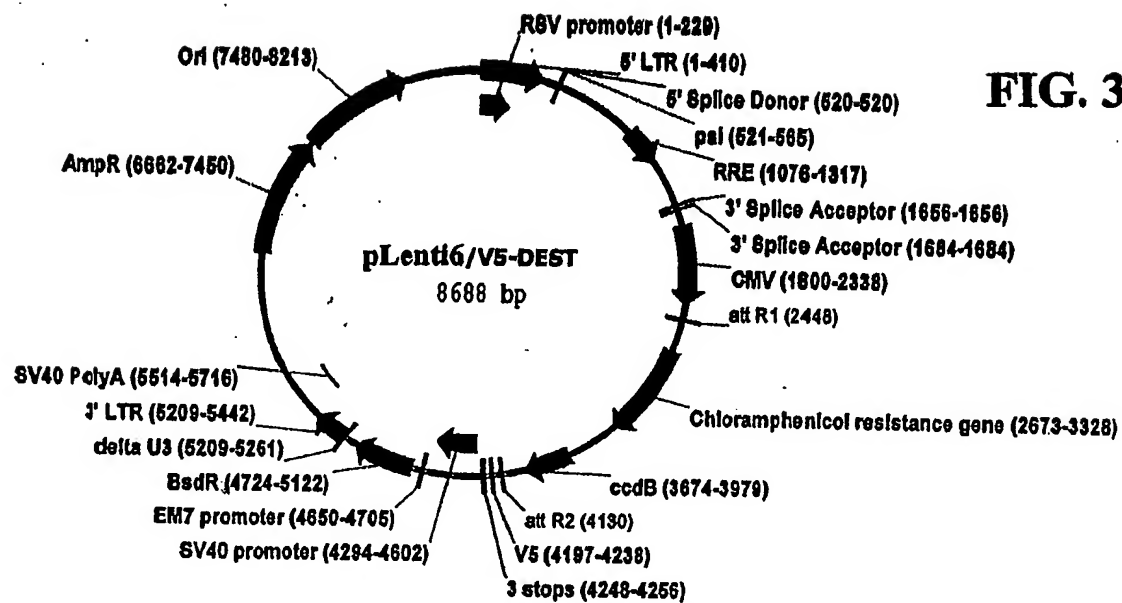
**FIG. 33**

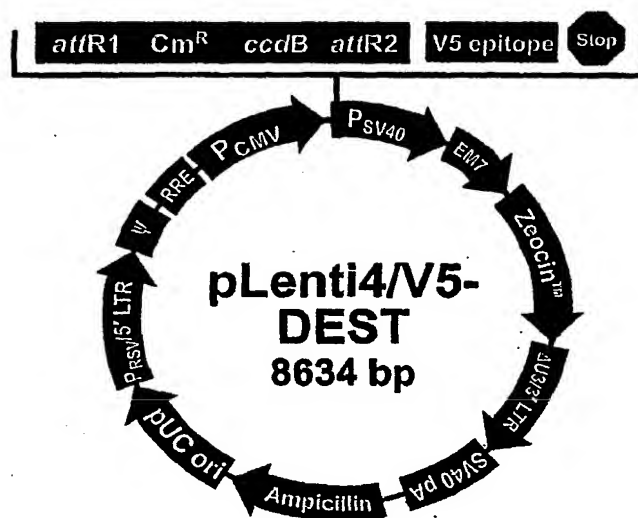


**FIG. 34**

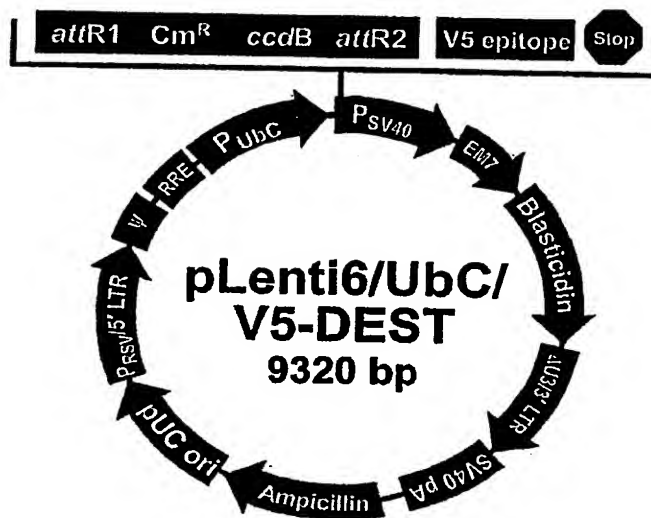


**FIG. 35**

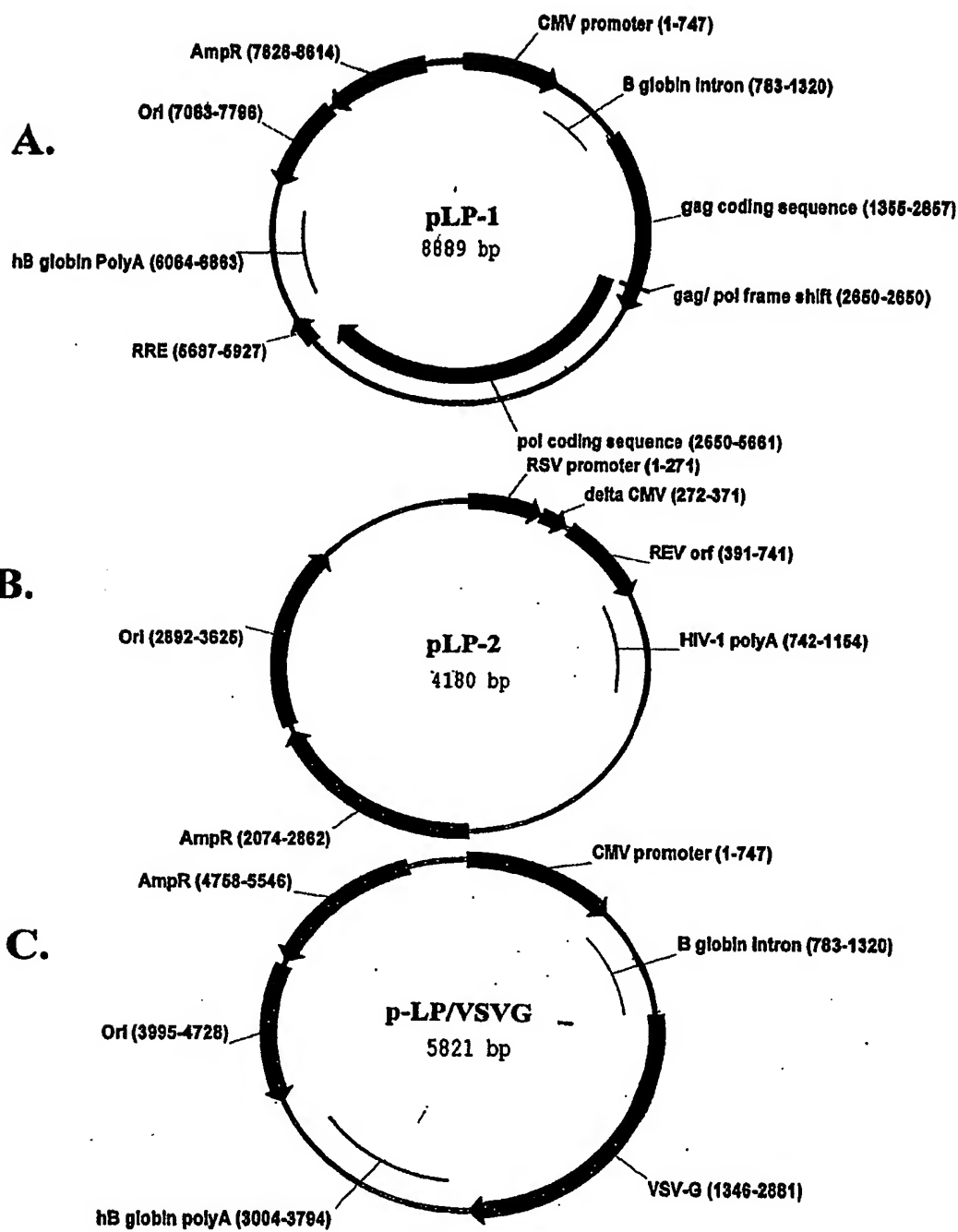




**FIG. 36C**



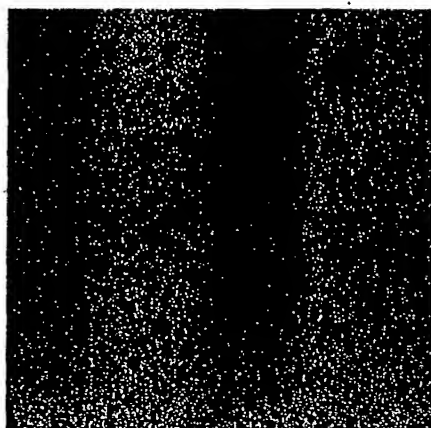
**FIG. 36D**



**FIG. 37**



**A.**



**B.**

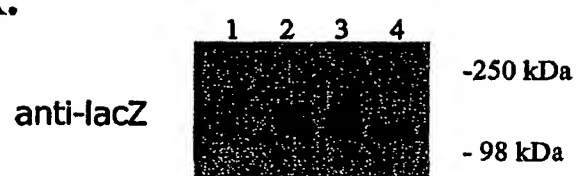
LR reaction [Bsd] in plate	DEST alone	DEST + CAT
No Bsd	24	320 <sup>1</sup> (12/24 = 50%) <sup>2</sup>
50 ug/ml Bsd	0	162 (24/24 = 100%) <sup>2</sup>

<sup>1</sup>see photo above

<sup>2</sup>percentage of correct colonies

**FIG. 38**

**A.**



**B.**



**FIG. 39**

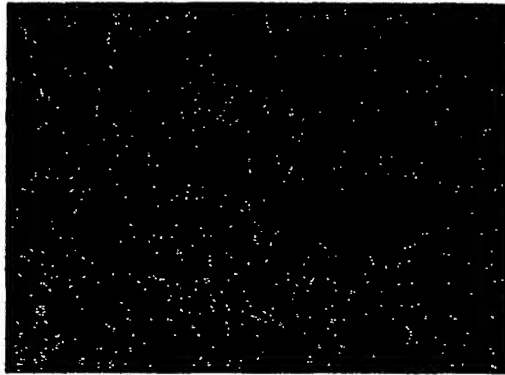
Examples of Production Titers (Bsd<sup>R</sup> cfu/ml)

	Empty	LacZ	GFP	CAT	PKC
Exp 1	$6 \times 10^6$	$5 \times 10^5$	$4 \times 10^6$	n.d.	n.d.
Exp 2	$3 \times 10^7$	$3 \times 10^5$	$6 \times 10^6$	$8 \times 10^6$	n.d.
Exp 3	$7 \times 10^6$	$6 \times 10^5$	$2 \times 10^6$	$1 \times 10^7$	$3 \times 10^6$
AVG	$1.4 \times 10^7$	$4.7 \times 10^5$	$4 \times 10^6$	$9 \times 10^6$	$3 \times 10^6$

n.d. = not determined

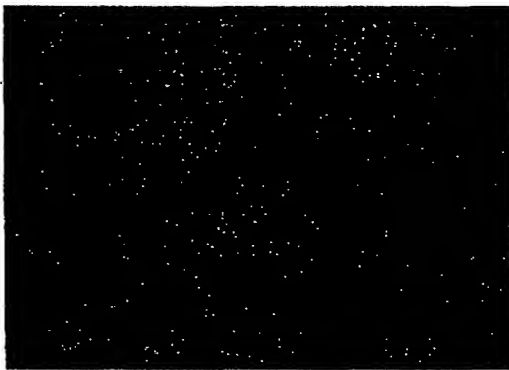
**FIG. 40**

**A.**



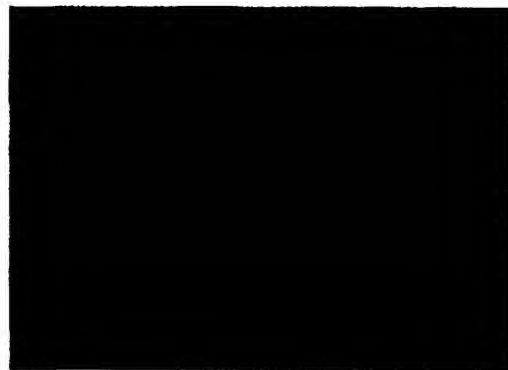
pLenti6/V5-GW/lacZ

**B.**



Brightfield

**C.**

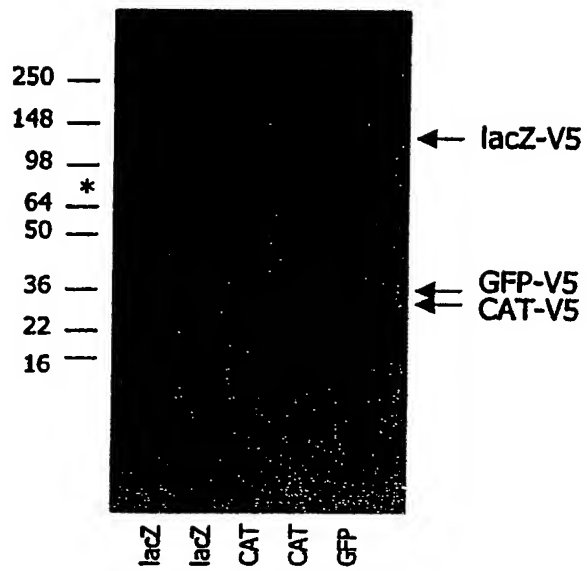


Fluorescent

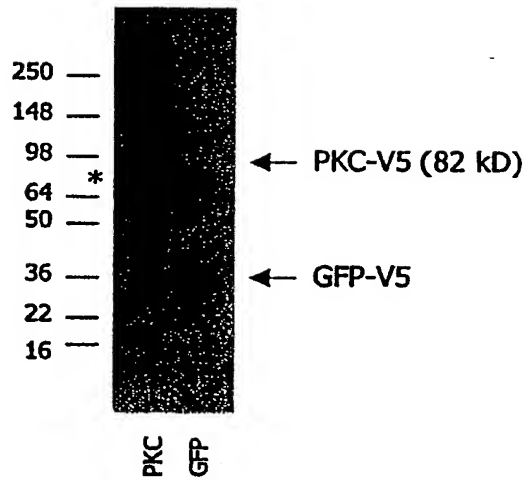
pLenti6/V5-dT/GFP

**FIG. 41**

**A.**

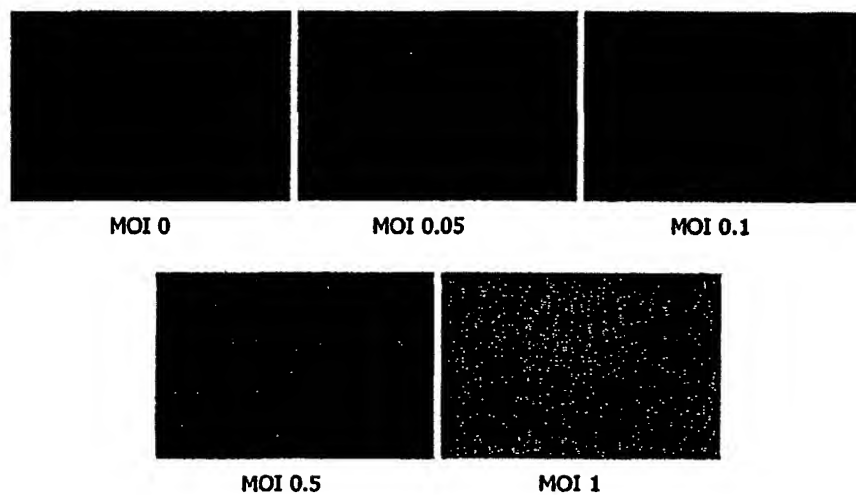


**B.**

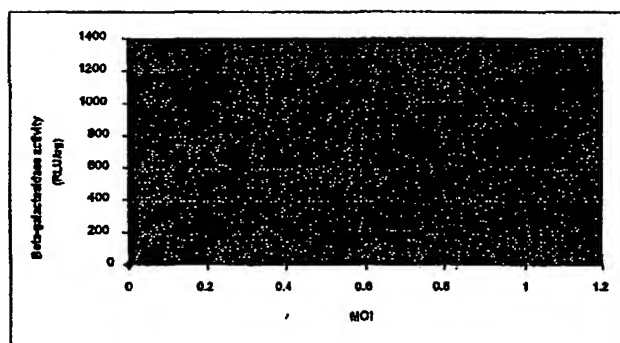


**FIG. 42**

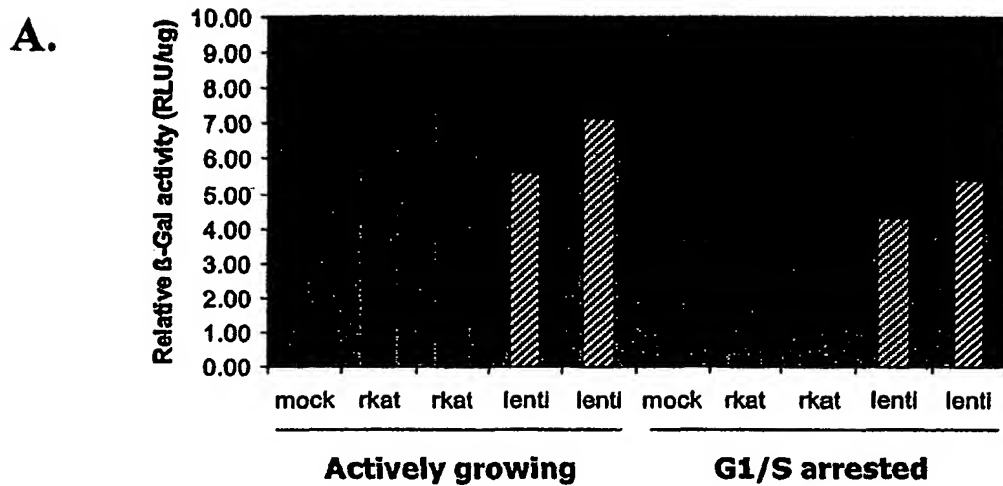
**A.**



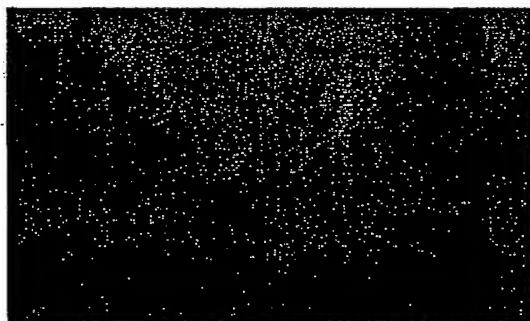
**B.**



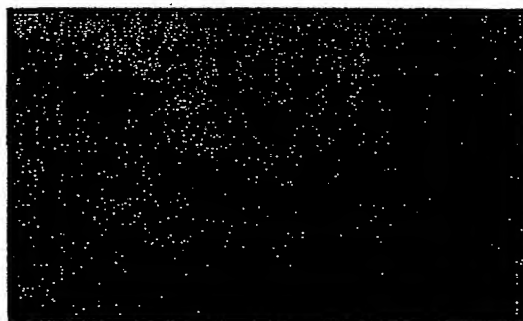
**FIG. 43**



**B.**



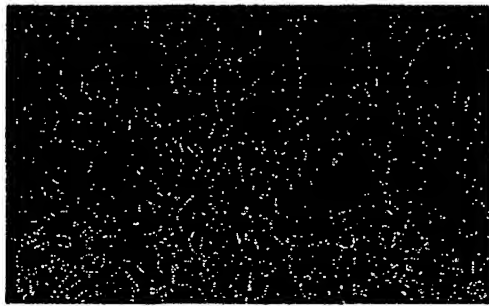
**rKAT6-lacZ retrovirus**



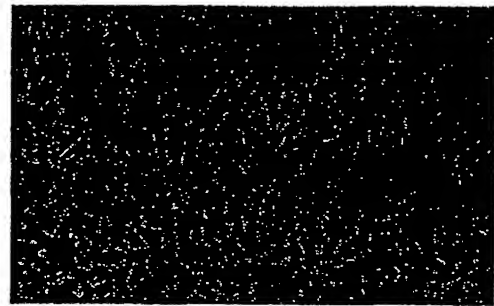
**pLenti6/V5-GW/lacZ**

**FIG. 44**

**A.**  
**10 days**

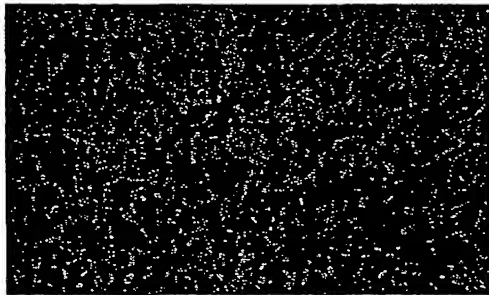


**rKAT6-lacZ retrovirus**

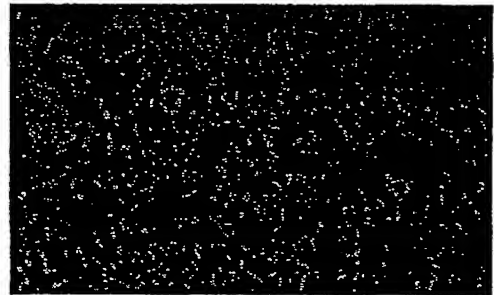


**pLenti6/V5-GW/lacZ lentivirus**

**B.**  
**6 weeks**



**rKAT6-lacZ retrovirus**



**pLenti6/V5-GW/lacZ lentivirus**

**FIG. 45**



FIG. 46A

2251 TCGTAACAAC TCCGCCCAT TGACGCAAT <sup>CAAT</sup> GGC CGGTAGG <sup>CMV forward priming site</sup> CGTGACGGT GGGAGGTCTA <sup>TATA</sup> TATAAGCAGA GCTCGTTTATG

2331 TGAACCGTCA GATCGCCTGG AGACGCCATC CACGCTGTTT TGACCTCCAT AGAAGACACC GACTCTAGAG GATCCACTAG

2411 TCCAGTGTGG TGGAATTCTG CAGATATCAA CAAGTTT <sup>2448</sup> ... Pro Ala Phe <sup>4130</sup> Leu  
 GTCTATAGTT GTTCRAACAT GTTT C TTG

4134 Tyr Lys Val Val Asp Ile Gln His Ser Gly Gly Arg Ser Ser Leu Glu Gly Pro Arg Phe Glu Gly  
 TAC AAA GTG GTT GAT ATC CAG CAC AGT GGC GGC CGC TCG AGT CTA GAG GGC CCG CGG TTC GAA GGT  
 TTT CAC CAA CTA TAG GTC GTG TCA CCG CCG GCG AGC TCA GAT CTC CCG GGC GCC AAG CTT CCA

4200 Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly \*\*\* \*\*\* \*\*\*  
 AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT TAG TAA TGA GTTT  
 TTC GGA TAG GGA TTC GGA GAG GAG CCA GAG CTA AGA TGC GCA TGG CCA ATC ATT ACT

V5 epitope V5 (C-term) reverse priming site

**FIG. 46B**

US forward printing site

2881 TTGGCGAGTG TGTTTTGTA AGTTTTTTAG GCACCTTTTG AAATGTAATC ATTTGGGTCA ATATGTAAIT TTCAGTGTTA

2961 GACTAGTAAA TTGTCCGCTA AATTCTGGCC GTTTTTGGCT TTTTGTGTTAG ACGAAGCTTG GTACCGAGCT CGGATCCACT

3041 AGTCCAGTGT GGTGGAATTC TGCAGATATC AACAGTTT ... Pro Ala Phe  
ACGTCTATAG TTGTTCAAAC ATGTTT C

3078 4762

atB1 atB2

4763 Leu Tyr Lys Val Val Asp Ile Gln His Ser Gly Gly Arg Ser Ser Leu Glu Gly Pro Arg Phe Glu  
TTG TAC AAA GTG GTT GAT ATC CAG CAC AGT GGC GGC CGC TCG AGT CTA GAG GGC CCG CGG TTC GAA  
TTC CAC CAA CTA TAG GTC GTG TCA CCG GCG GCG AGC TCA GAT CTC CCG GGC GCC AAG CTT

4829 Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly \*\*\* \*\*\* \*\*\*  
GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT GAT TCT TCT ACG CGT ACC GGT TAG TAA TGA GTTT  
CCA TTC GGA TAG GGA TTC GGA GAG GAG CCA CAG CTA AGA TGC GCA TGG CCA

V5 epitope V5 (C-term) reverse priming site

# FIG. 46C

1786 <sup>5' end of UbO promoter</sup>  
 CGGATCTGGC CTCCGCGCGG GGTTTTGCGG CCTCCGCGGG GCGCCGCCCT CCTCACGGCG AGCGCTGCCA CGTCAGACGA  
 1876 AGGGCGCAGG AGCGTCTGA TCCT<sup>Sp 1</sup>CCGCCGGGAGCTCA GGACAGCGGC CCGCTGCTCA TAAGACTCGG CCTTAGAACC  
 1956 CCAGTATCAG CAGAAGGACA TTTAGGACG GGA<sup>Sp 1</sup>CTGGGT GACTCTAGGG CACTGGTTT CTTCCAGAG AGCGGAACAG  
 2036 GCGAGGAAAA GTAGTCCCTT CTGCGGATT CTGCGGAGGG ATCTCGTG<sup>Sp 1</sup>GCGCGGAAC GCGATGATT ATATAAGGAC  
 2116 <sup>TATA box</sup>  
 GCGCCGSGTG TGGCACAGCT <sup>Start of Transcription</sup>  
AGTTCGTCG CAGCCGGGAT TTGGGTCGG GTTCTGTIT GTGGAICGCT GTGATCGTCA  
 2196 <sup>Exon 1</sup>  
 CTTGTGAGT AGCGGGCTGC TGGGCTGGCC GGGGCTTTCG TGGCGCCGG GCGCTCGGT GGGACGGAAG CGTGTGGAGA  
 2276 GACGCCAAG GGTGTAGTC TGGGTCGCG AGCAAGGTTG CCTGAACTG GGGTTGGGG GGAGCGCAGC AAAATGGCGG  
 2356 CTGTTCCGA GTCTGAATG GAAGACGCTT GTGAGGCGGG CTGTGAGTC GTTGAACAA GGTGGGGGGC ATGGTGGGG  
 2436 GCAAGAACCC AAGGCTTGA GGCCTTCGT AATGCGGAA AGCTCTTATT CCGGTGAGAT GGGCTGGGGC ACCATCTGG  
 2516 GACCCGACG TGAAGTTGT CACTGACTGG AGA<sup>Sp 1</sup>CTGGT TTGTCGTCTG TTGCGGGGC GGCAGTTATG CGGTGCCGT  
 2596 GGGCAGTGA CCGTACCTT TGGGAGCGG CCGCTCGTC GTGTCGTGAC GTACCCGTT CTGTGGCTT ATAATGCAGG  
 2676 GTGGGGCCAC CTGCGGTAG GTGTGGGTA GGTCTTCTC CGTCGCAGGA CGCAGGGTTC GGGCTAGGG TAGGCTCTCC  
 2756 TGAATCGACA GCGCGCGGAC CTCGTGTGAG GGGAGGGATA AGTGAGCGT CAGTTCTTT GGTGGTTTT ATGTACCTAT  
 2836 CTTCTAAGT AGCTGAAGCT CCGGTTTGA ACTATGCGCT CGGGGTGGC GAGTGTGTT TGTGAAGTT TTAGGCACC  
 2916 <sup>UB Forward priming site</sup>  
 TTTTGAATG TAATCATTTG GGTCAATG TAATTTCAG TGTAGACTA GTAAATGTC CGCTAAATC TGGCCGTTT  
 2996 <sup>5' end of Intron 1</sup>  
 TGGCTTTTT GTTAGCGAA GCTTGG....  
 5' end of Exon 2

FIG. 47

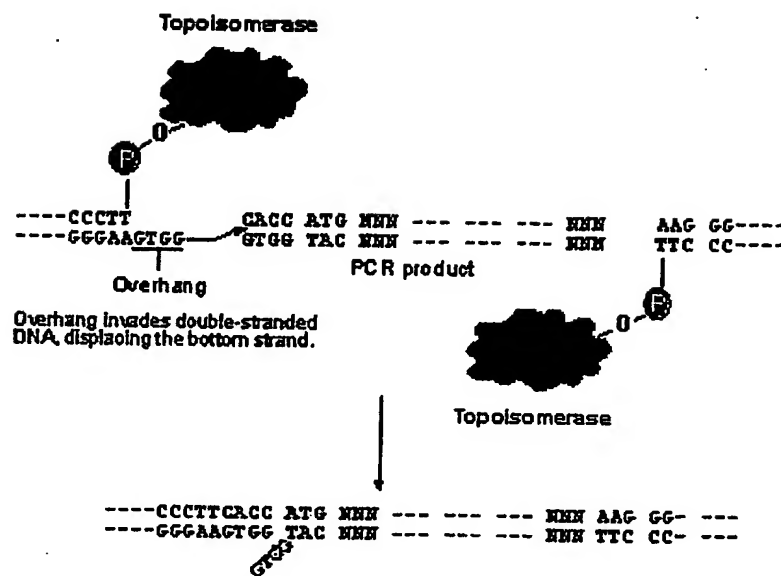
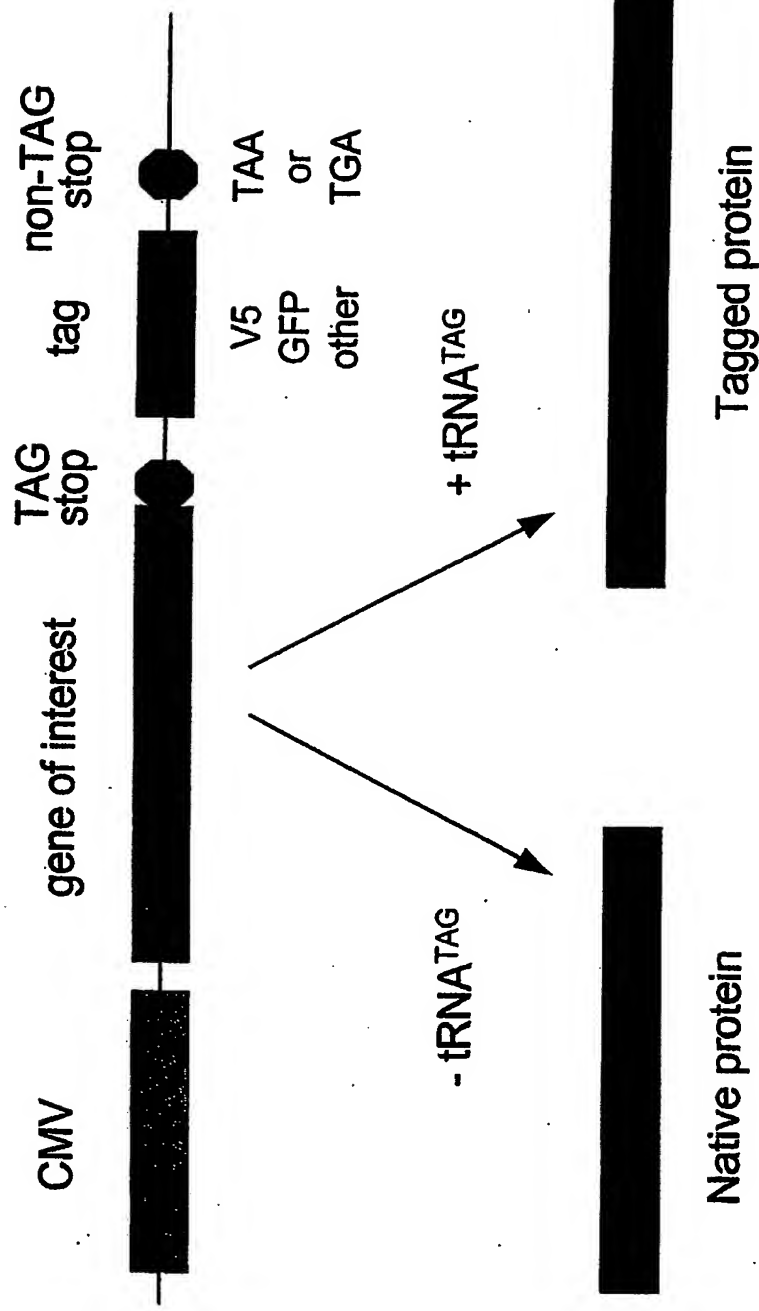


FIG. 48

2251 TCGTAACAAC TCCGCCCCAT TGACGCAAAAT GGGCGGTAGG CGTGACGGT GGGAGGTCTA TATAAGCAGA GCTCGTTTAG  
 CAAT CMV forward priming site TATA 3' end of CMV promoter  
 2331 TGAACCGTCA GATCGCCTGG AGACGCCATC CACGCTGTTT TGACCTCCAT AGAAGACACC GACTCTAGAG GATCCACTAG  
 Transcriptional start BamHI SpeI  
 2411 TCCAGTGTGG TGGAATTGAT CCCTT ACC ATG ... AAG GGC TCG AGT CTA GAG GGC CCG CGG TTC GAA GGT  
 BstXI XhoI ApsI SacII SfiI  
 CTA GGAAG TGC TAC ... TTC CCA GTT CTG  
 Lys Gly Ser Ser Leu Glu Gly Pro Arg Phe Glu Gly  
 V5 epitope V5(C-term) reverse priming site  
 2476 AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT TAG TAA TGA GTTGGAA  
 Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly \*\*\* \*\*\* \*\*\*  
 2541 TTAATTCTGT

**FIG. 49**





**FIG. 50**

FIG. 51A

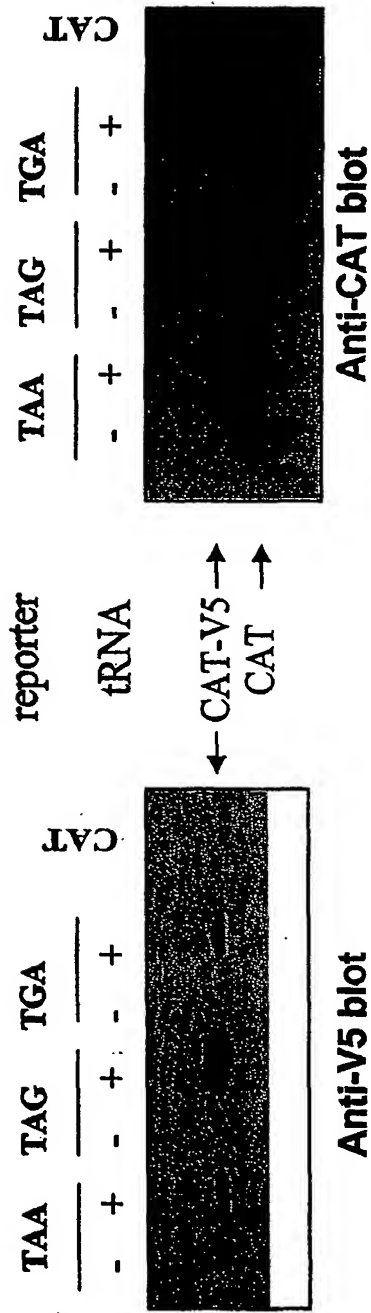
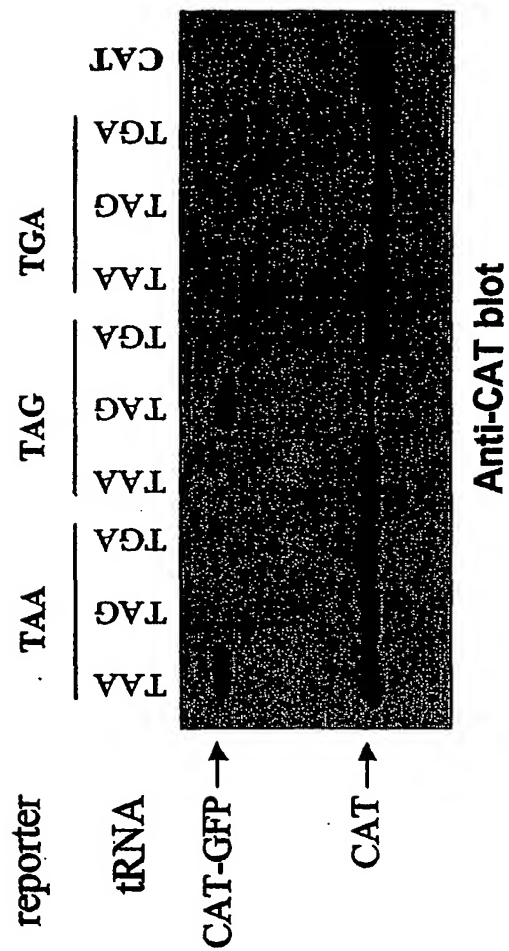
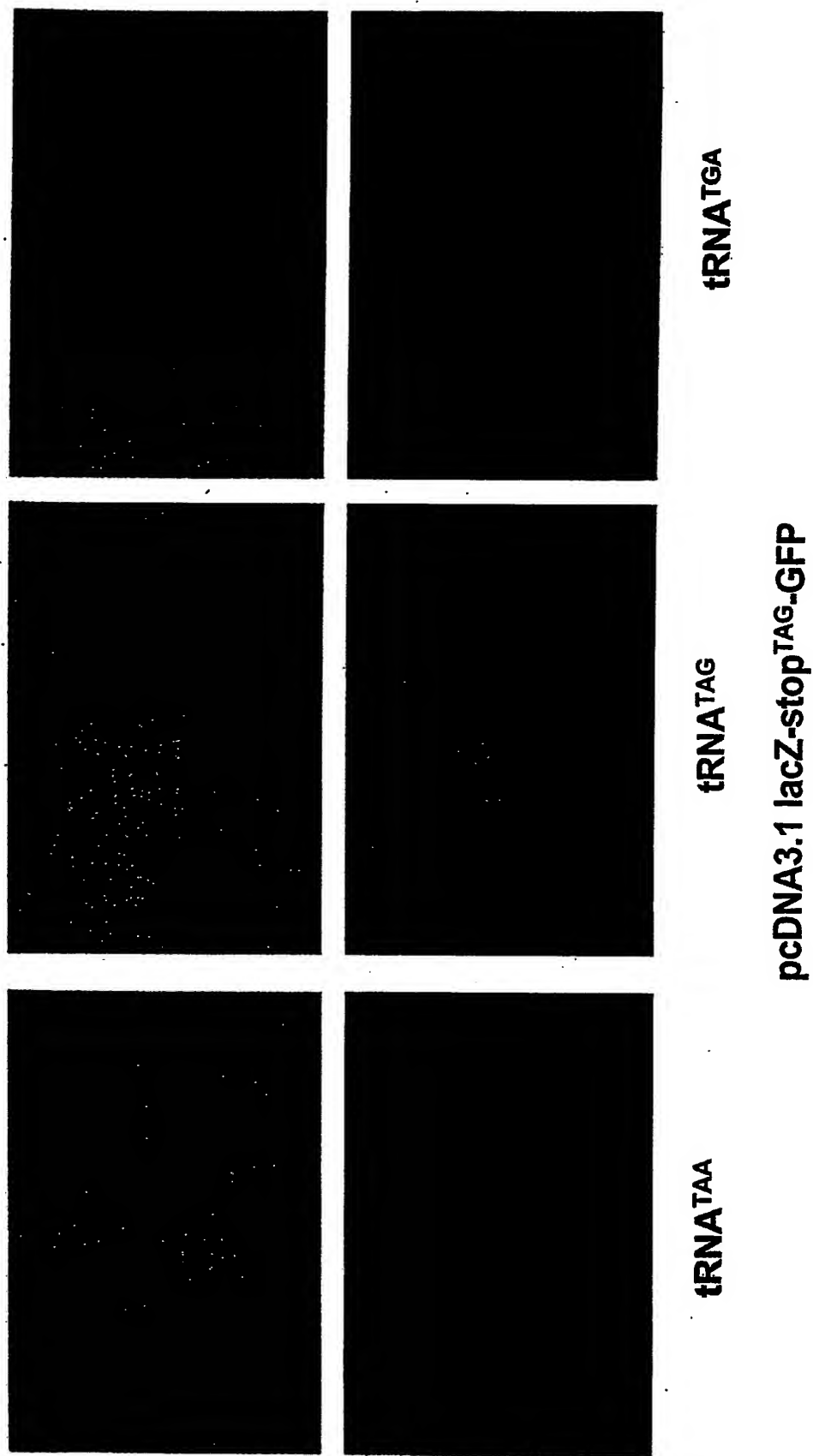


FIG. 51B



FIGS. 51 A-B

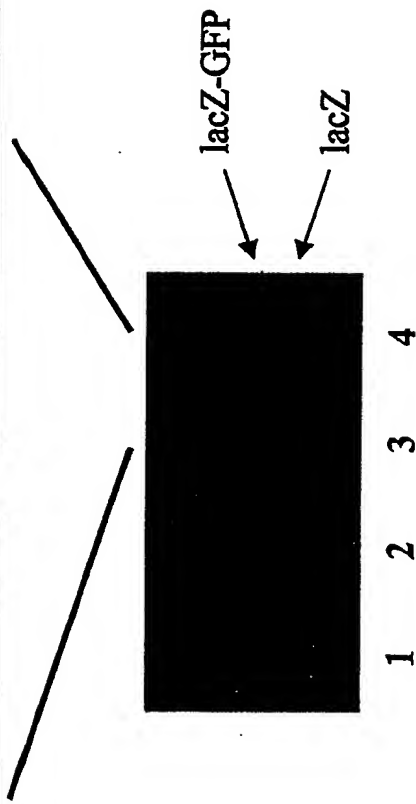
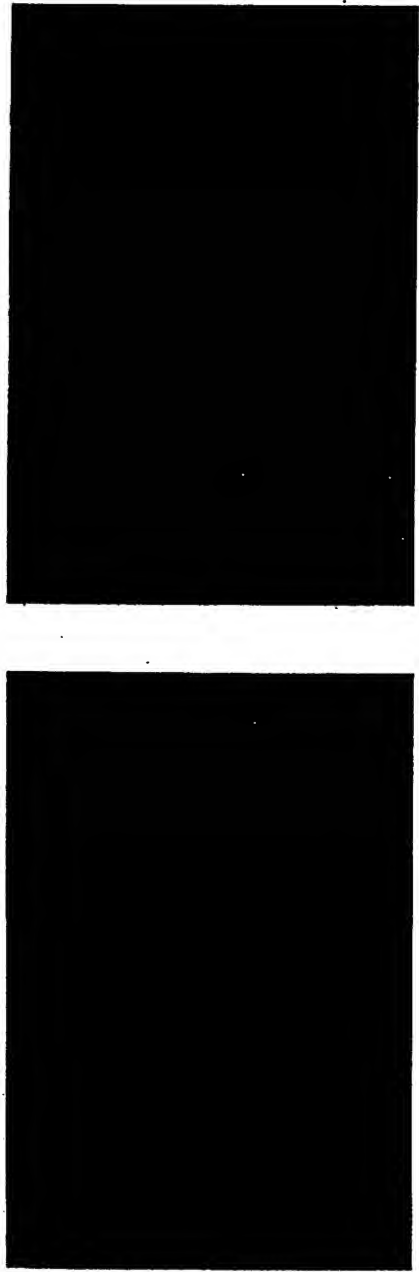




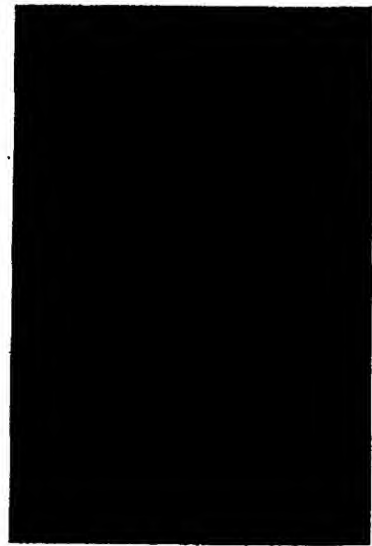
**FIG. 52**

**Monomer**

**8-mer**



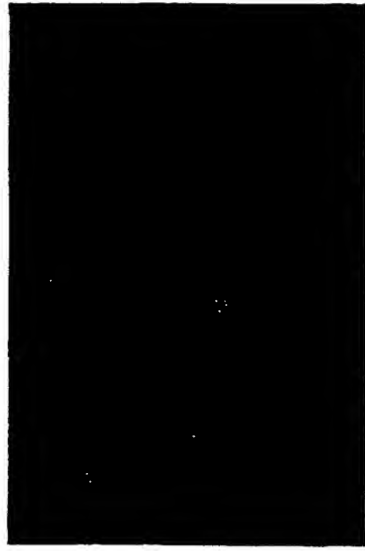
**FIG. 53**



ORF6 (BC003357)

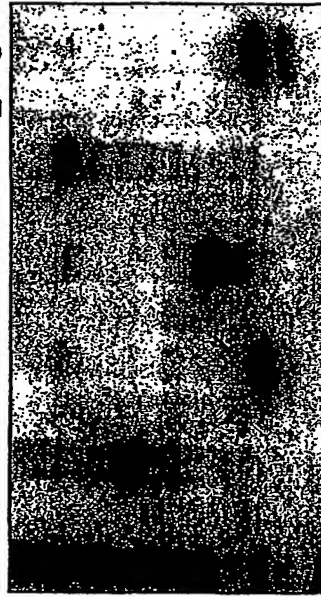


ORF7 (BC000997)



ORF12 (BC000141)

tRNA:		lacZ		mock	GFP-V5
12	6	7	lacZ		
-	+	-	+	-	+

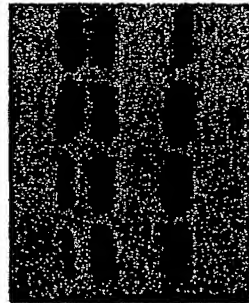


Anti-V5 blot

FIG. 54

**FIG. 55A**

MOI: 19 51 77 192



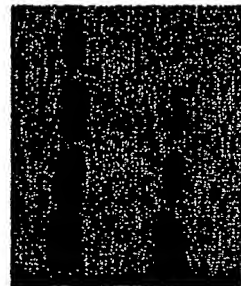
\*

% suppression: 13 30 51 60

STABLE GOI

**FIG. 55B**

MOI: 19 38 77 192



← lacZ-GFP →

← lacZ →

% suppression: 63 71 76 75

TRANSIENT GOI



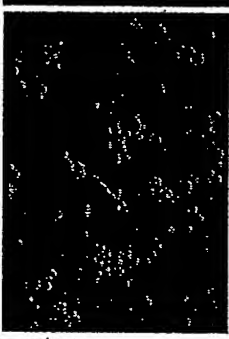


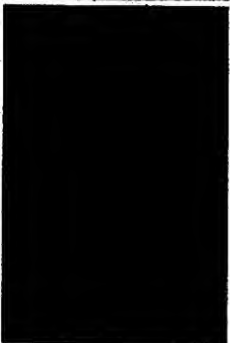




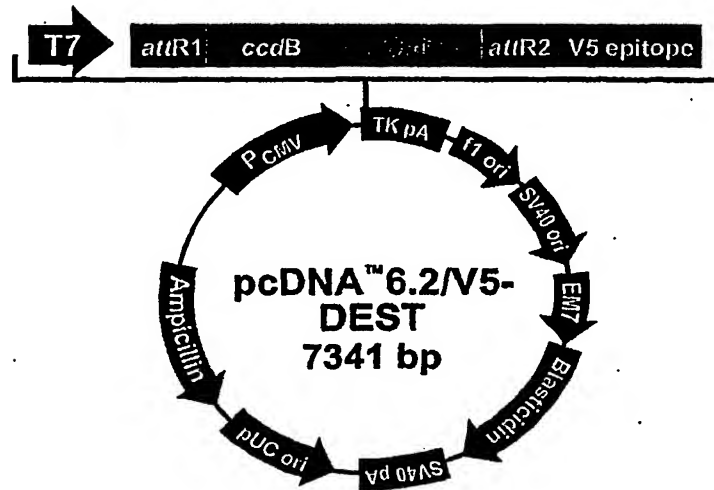
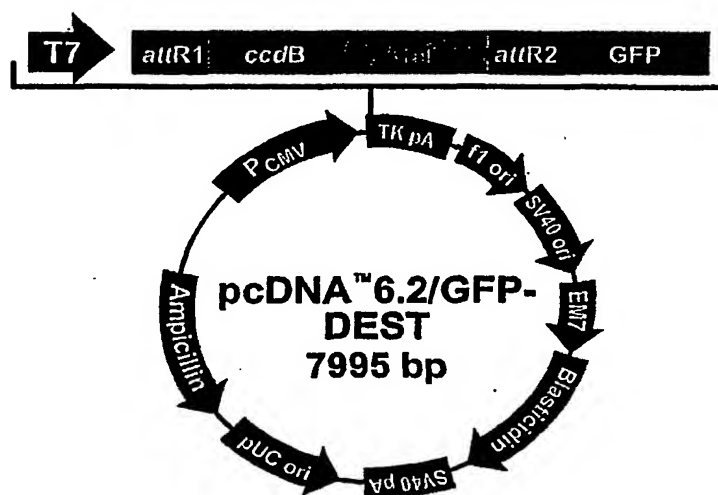
BHK-21	CHO-S	COS-7	HeLa	HT1080
				
				

FIG. 56

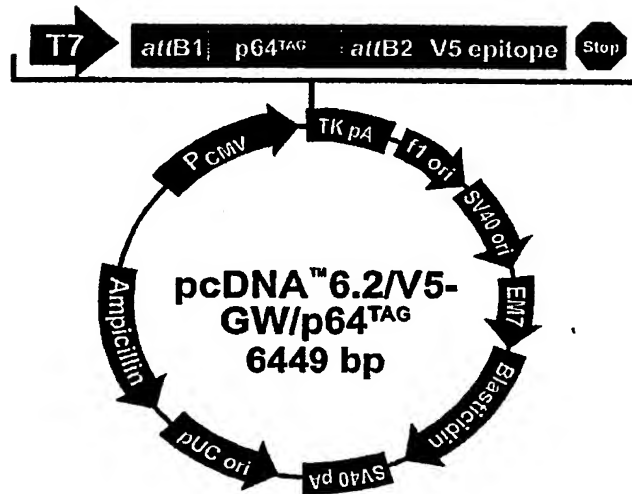
**FIG. 57**



**FIG. 58**

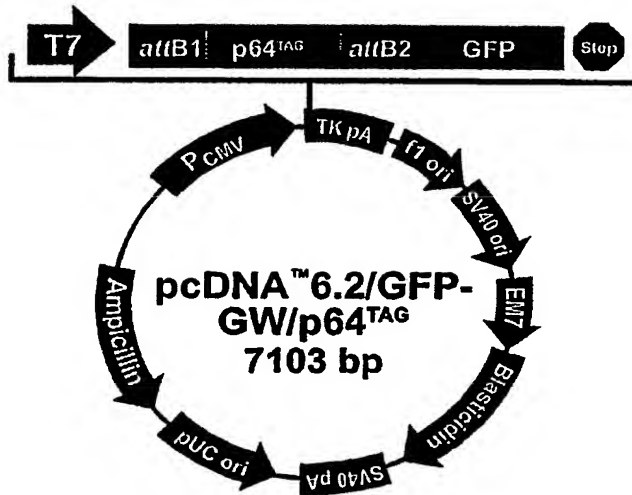


**FIG. 59**





**FIG. 60**



# FIG. 61

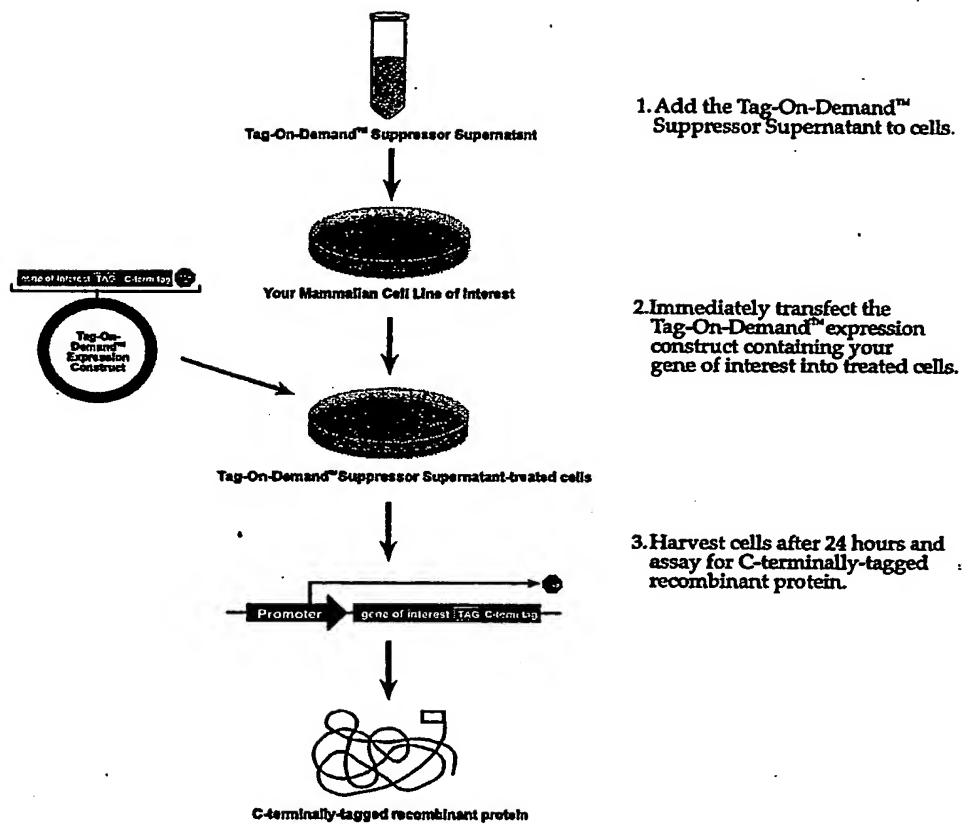
A.

CAAT TATA 3' end of CMV promoter Putative transcriptional start  
 771 CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA  
 T7 promoter/priming site  
 841 CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGT TAAGCTATCA  
 918 attB 1 3161 attB 2  
 911 ACAAGTTT TGTTCAAACA TGTTT C TTG TAC AAA GTG GTT TTT CAC CAA  
 Pro Ala Phe Leu Tyr Lys Val Val  
 V5 epitope V5 reverse priming site  
 3177 GAT CTA GAG GGC CCG CGG TTC GAA GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC  
 Asp Leu Glu Gly Pro Arg Phe Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu  
 3234 GAT TCT ACG CGT ACC GGT TAG TAA TGA GTTTAAACGG GGGAGGCTAA CTGAAACACG  
 Asp Ser Thr Arg Thr Glv \*\*\* \*\*\* \*\*\*

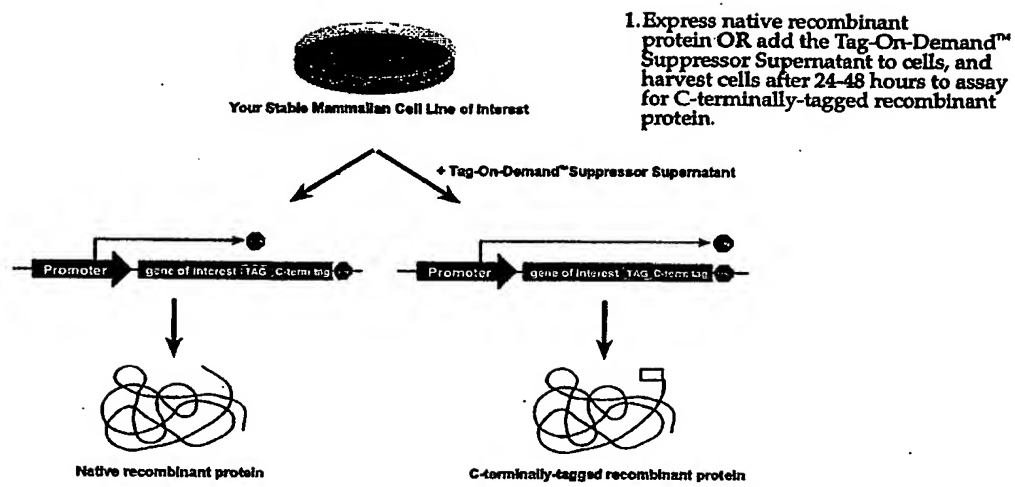
B.

CAAT TATA 3' end of CMV promoter Putative transcriptional start  
 771 CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA  
 T7 promoter/priming site  
 841 CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGT TAAGCTATCA  
 918 attB 1 3161 attB 2  
 911 ACAAGTTT TGTTCAAACA TGTTT C TTG TAC AAA GTG GTT TTT CAC CAA  
 Pro Ala Phe Leu Tyr Lys Val Val  
 Green Fluorescent Protein (cycle-3 GFP)  
 3177 GAT CTA GAG GGC CCC GCG GCT AGC AAA GGA GAA GAA CTT TTC ACT GGA GGT GTC CCA  
 Asp Leu Glu Gly Pro Ala Ala Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro  
 3234 ATT CTT GTT GAA TTA GAT GGT GAT GTT AAT GGG CAC AAA TTT TCT GTC AGT GGA GAG  
 Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu  
 GFP reverse priming site  
 3291 GGT GAA GGT GAT GCT ACA TAC GGA AAG CTT ACC CTT AAA TTT ATT TGC ACT ACT GGA  
 Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly  
 3348 AAA CTA CCT GTT ...  
 Lys Leu Pro Val ...

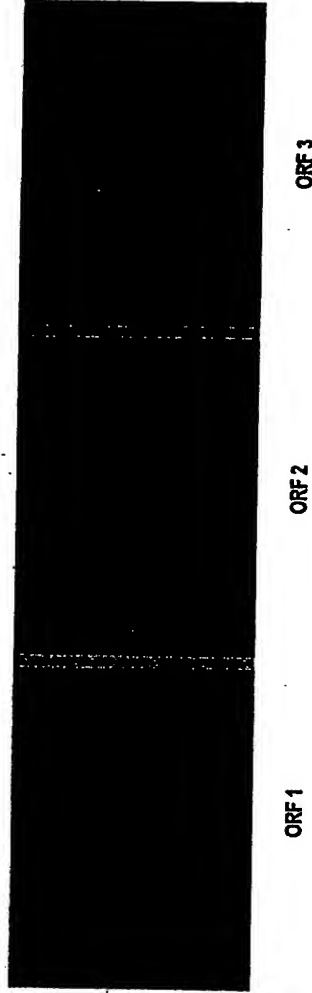
**FIG. 62**

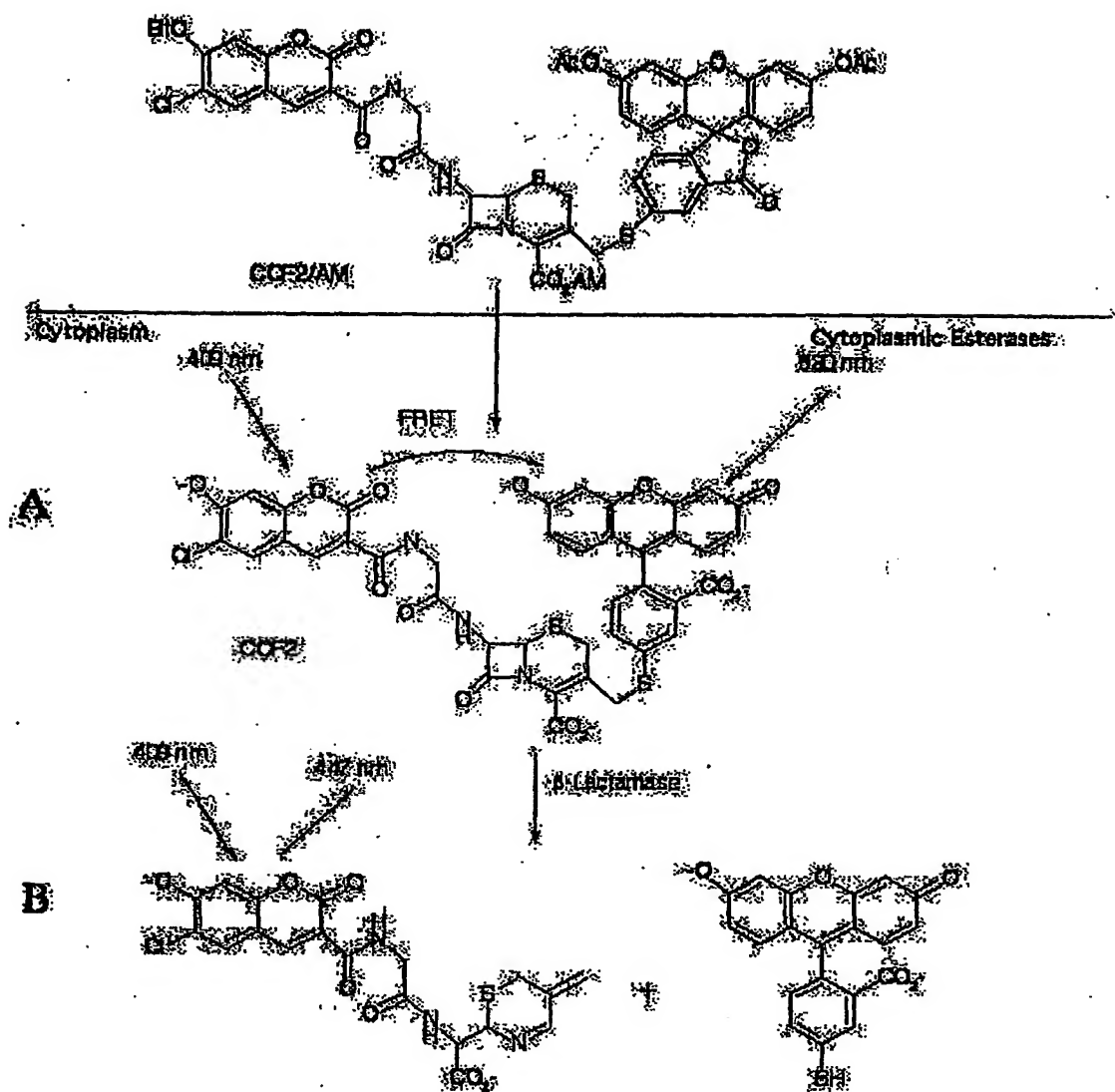


**FIG. 63**



**FIG. 64**





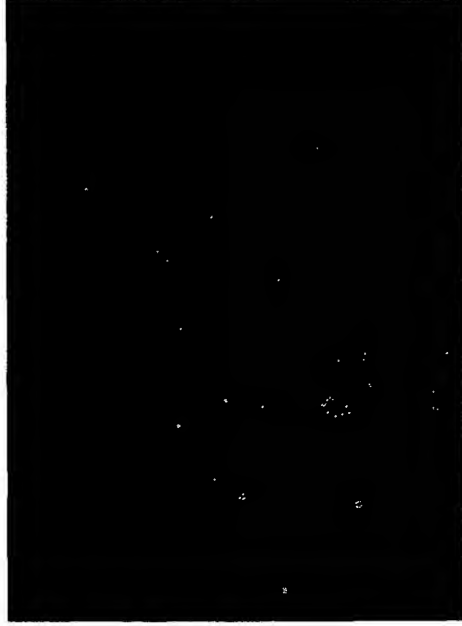
**FIG. 65**

**Fig. 66**

**Sequential method**



**Simultaneous method**



**Fluorescent**

**Brightfield**

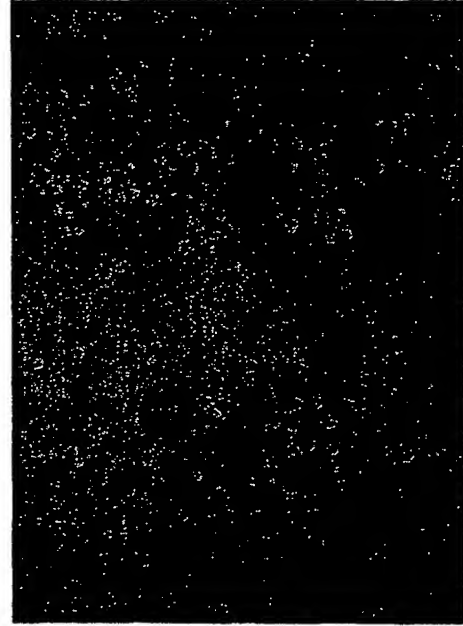
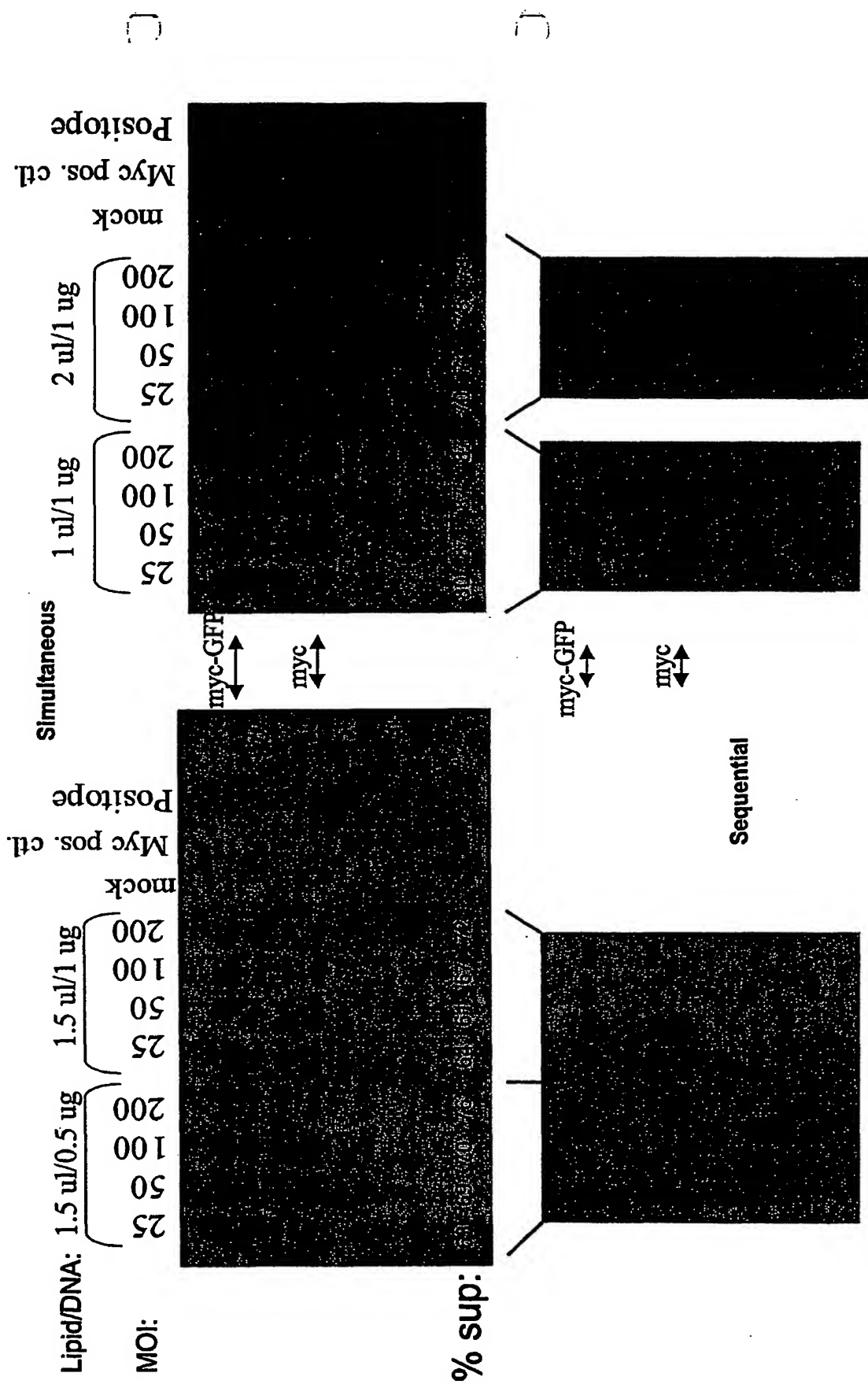


FIG. 67





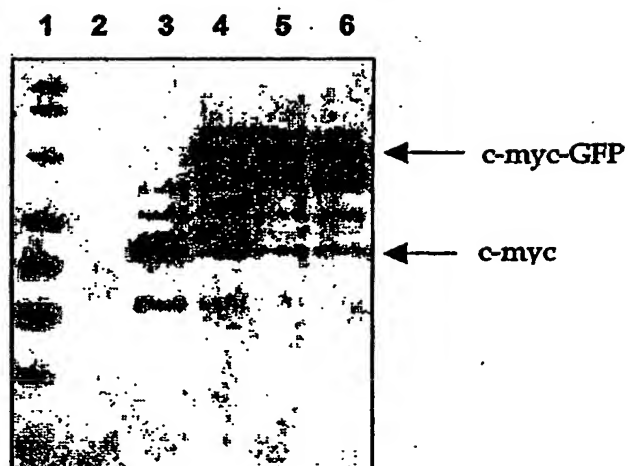


Fig. 6b

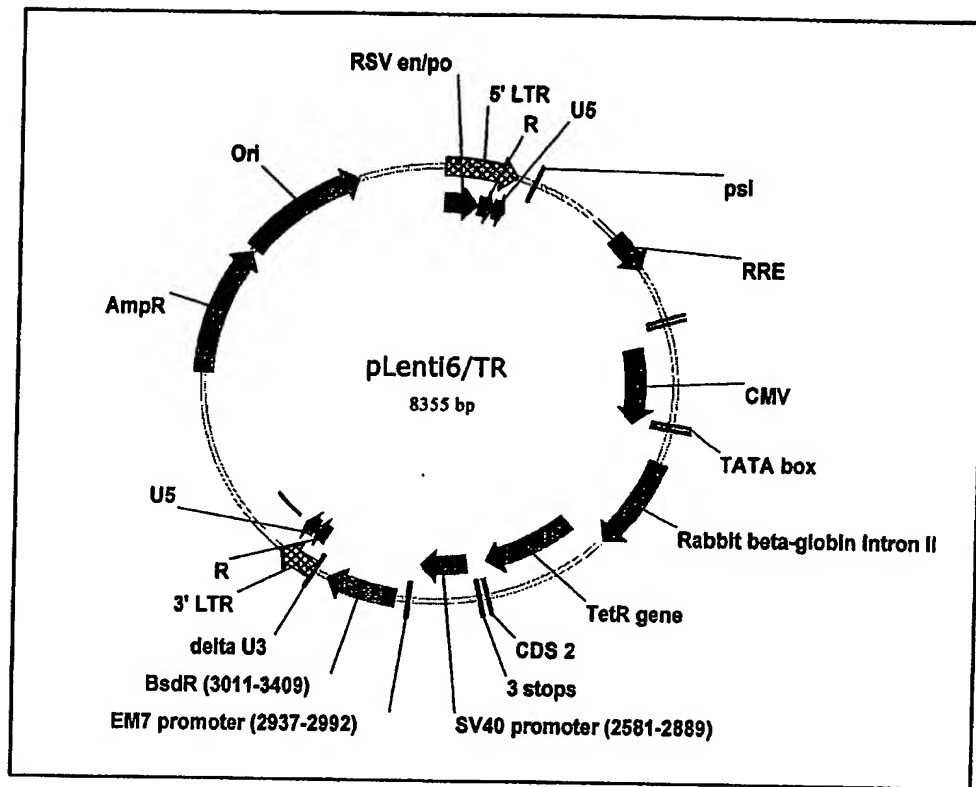


FIG. 69

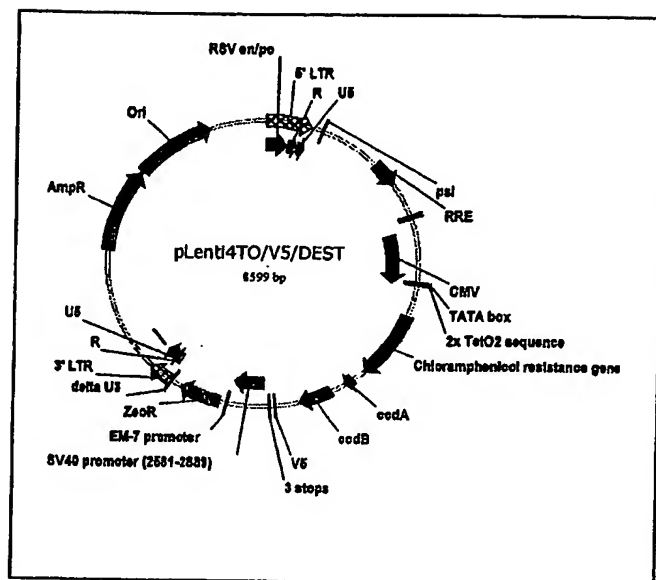


FIG. 70A

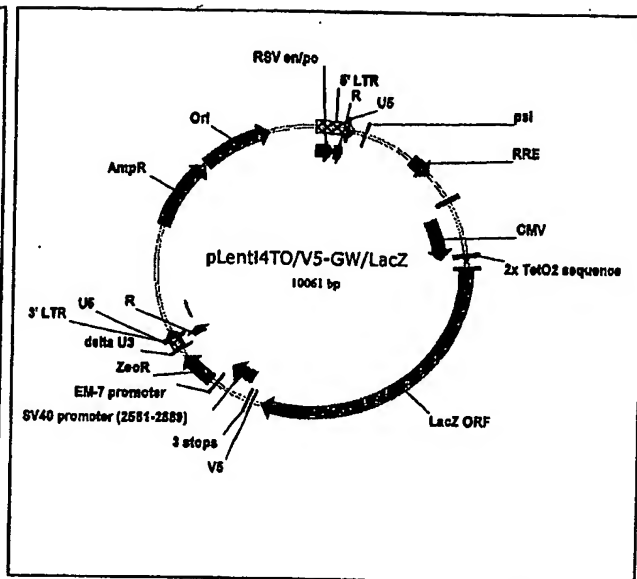
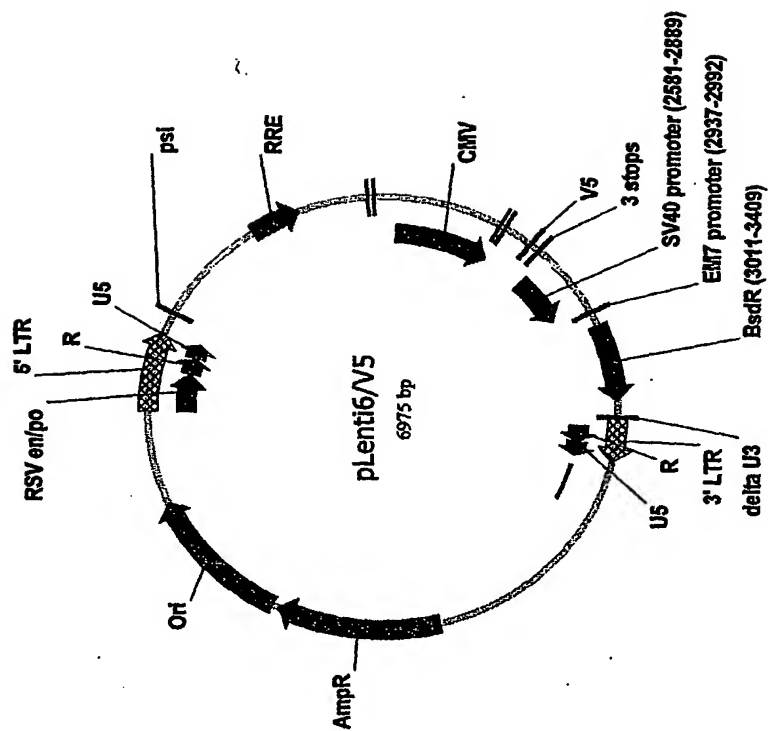
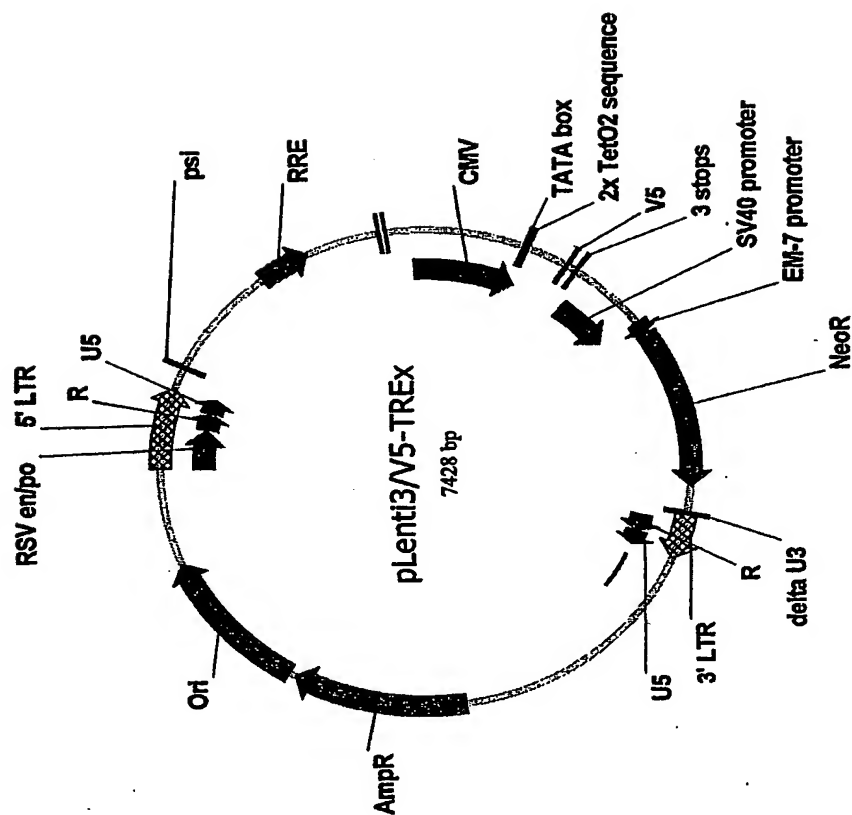


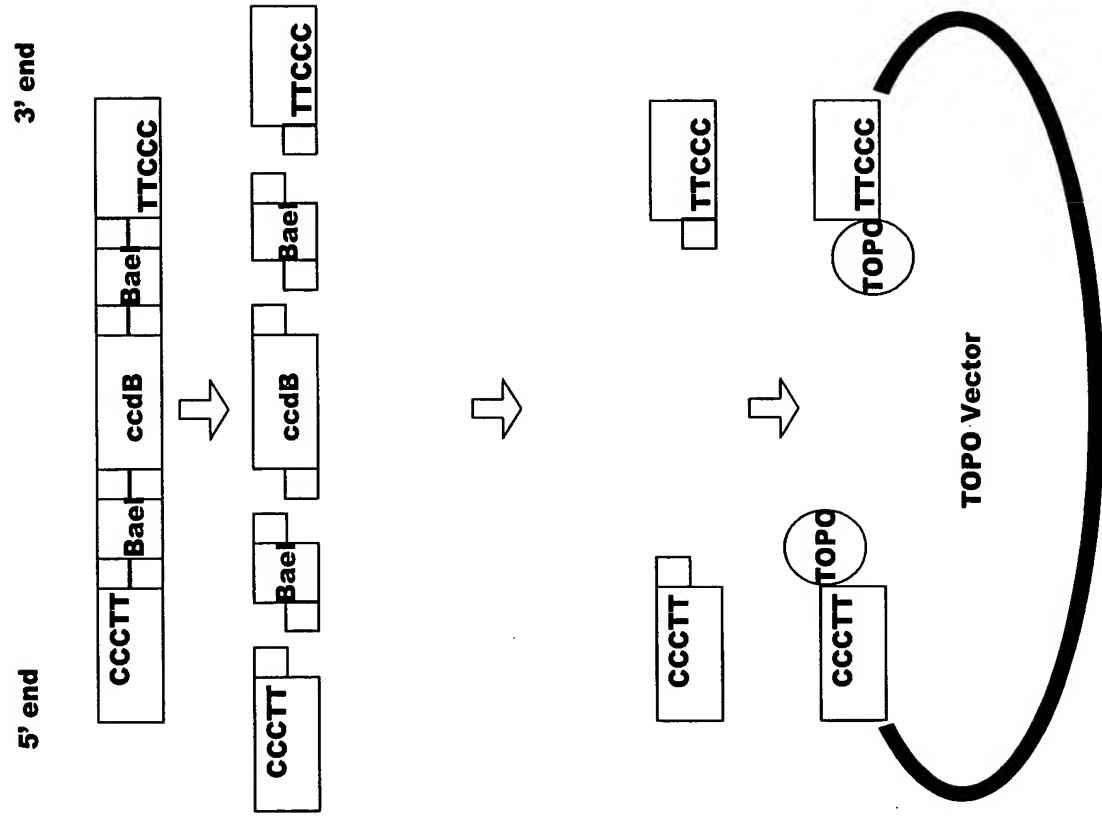
FIG. 70B



**FIG. 71**



**FIG. 72**



**FIG. 73**